XI. *The Giant Nerve Cells and Fibres of* Halla parthenopeia.

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(Received May 30,—Read June 18, 1908.)

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1. INTRODUCTION.

THE giant nerve fibres, which form so prominent a feature in the transverse section of the nerve cord of many Annelids, were first observed in these animals by CLAPARÈDE in 1861, who, however, regarded them as canals. They were first recognised as nervous elements—"riesige dunkelrandige Nervenfasern"—by LEYDIG in 1864. Since then their nervous nature has been almost alternately affirmed and denied, and many widely divergent views have been advanced regarding their morphology and function. The connection of giant fibres with certain giant nerve cells was first shown in the case of *Halla parthenopeia*, by SPENGEL, in 1881. Although many other workers have investigated these elements, information is still lacking regarding several fundamental points of their structure. For instance, nothing is known regarding the neurofibrillæ of the giant cells, and although these conducting elements have been seen by five observers in the giant fibres of earthworms, there is a striking difference in their accounts: two of them refer to the presence of several neurofibrillæ, while the others describe or figure only a single fibril in each giant fibre. Further, no information is available regarding the place and mode of origin of these neurofibrillæ or their relations to other nerve elements. This defect is, no doubt, due largely to the difficulties attending the investigation of these remarkable cells and fibres; indeed, the failure of the methods usually adopted for staining nerve cells and fibres in other animals, to disclose nervous elements in the giant cells and fibres, has been held, for instance, by VON LENHOSSÉK and RETZIUS, to disprove their nervous nature.

The present investigation was commenced in 1900 with the view of determining the character and arrangement of the neurofibrillæ of the giant cells and fibres and the relations of these elements to the other elements of the nerve cord.

There are considerable differences in the structural details, the relative proportions, and the arrangement of the giant cells and fibres in various Annelids, but after a prolonged trial of several genera and species, *Halla parthenopeia** was finally

* *Halla parthenopeia*, A. COSTA, and *Aglaurides fulgida*, SAVIGNY, are large nereidiform Polychæta. The former is placed in the family Lysaretidæ, and the latter in the family Eunicidæ. Both occur in shallow

chosen as presenting the most favourable material for the study of these remarkable elements of the nervous system. This choice was made principally on account of the large size of both the cells and fibres and the clearness of the connection between the giant cell and its giant fibre. Moreover, each giant fibre of *Halla* is connected with only one giant cell, whereas in *Arenicola*, the earthworms, and many other Annelids, each giant fibre is composed of the conjoined processes of several or many giant cells; further, there are none of those complex anastomoses between the various giant fibres of *Halla* such as are present in *Arenicola* and some other Polychætes, and in many Oligochætes. Consequently the relations of the giant fibres are less complex in *Halla* than in most other Annelids in which such fibres are present, a fact which, taken along with the large size of the giant nerve elements in this worm, indicates that *Halla* is the most favourable starting point for the study of these striking but refractory structures. The results have been confirmed by an examination of the corresponding structures in *Aglaurides fulgida** in which the neurofibrillæ are especially well seen.

2. HISTORICAL ACCOUNT OF THE GIANT NERVE CELLS AND GIANT NERVE FIBRES OF ANNELIDS.

The first record of the occurrence of a giant fibre in Annelids is in CLAPARÈDE'S description (1861, pp. 75 and 104) of the nerve cord of the Oligochætes, *Pachydrilus* and *Clitellio*, in which the fibre was mistaken for a thin axial cylinder. In a further account (1862, pp. 225, 226) CLAPARÈDE described the nerve cord of these worms as consisting of a cortical substance (that is, the ordinary nerve fibres) and a bundle of central fibres, generally large, each composed of a folded envelope and of a less refringent axial substance. The fibre which occupied the middle of the bundle had a thicker and more obviously folded envelope than the others, and was consequently more distinct; it was this fibre which was at first mistaken for an axial canal. CLAPARÈDE noted that these fibres run, without branching, from one end of the nerve cord to the other, and that he had not succeeded in recognising any connection between them and the relatively small cells of the anterior ganglia.

KEFERSTEIN (1862, p. 125) was the first to observe a giant fibre in a Polychæte; in the nerve cord of *Capitella rubicunda* he saw a "central canal," and in the following year CLAPARÈDE (1863, p. 27) also saw this "Axen-canal" in the same

water in warmer seas. Neapolitan specimens of *Halla* attain a considerable length. I have had a dozen which, when alive and extended, measured from 50 to 70 cm. in length; one measured 84 cm. and another attained the great length of 110 cm. when extended. I am indebted to Mr. CYRIL CROSSLAND for all the specimens of *Aglaurides* used in this work. They were collected by him in the neighbourhood of Zanzibar and in the Red Sea. *Aglaurides fulgida* is smaller than *Halla parthenopeia*. The largest Red Sea specimen of the former seen by Mr. CROSSLAND measured 48 cm. in length when alive, the largest of the preserved ones in my hands (a specimen from Zanzibar) is 31·5 cm. long.

* See preceding footnote.

animal, and stated that while in many Lumbricids the corresponding structure may be regarded as a thicker fibre, it is scarcely possible, in the case of *Capitella*, to doubt that it is a canal.

The giant fibres were first recognised as, and designated, nervous elements by LEYDIG in 1864. Since his account there have been many interpretations advanced regarding the nature of the giant fibres and of the giant cells with which they were later found to be connected. Instead of considering this voluminous literature in strict chronological order it will, I think, be more convenient and satisfactory to classify the memoirs into groups, each dealing with a particular view of the nature of these elements.

I. THE GIANT FIBRES REGARDED AS TRUE NERVE FIBRES.

LEYDIG (1864, p. 154) described the giant fibres which he found in the nerve cord of several Lumbricids* as "riesige dunkelrandige Nervenfasern,"† and pointed out that they always lie in the mid-dorsal line of the cord (that is, they are not axial as stated by CLAPARÈDE), and that they react to stains like the nerve fibres of vertebrates. He concluded that their contents, in the form of a pale band, correspond to the axis cylinder, and their wall to the medullary sheath of an ordinary medullated nerve fibre of a vertebrate, but the giant fibres have no envelope corresponding to the sheath of Schwann. He traced the giant fibres, three of which were present in

* He also described giant nerve fibres in the cord of *Lumbriculus variegatus* (p. 171, Taf. 4, fig. 6), *Stylaria proboscidea* (p. 171, Taf. 4, fig. 5), and *Enchytræus galba* and *latus* (p. 174).

† LEYDIG'S interpretation of these tubes as nervous, though new as regards its application to Annelids, was not a new conception. For many years the nerve fibres of Vertebrates had been considered to be tubular, and as far back as 1833 EHRENBERG (1836, p. 720) had observed in the nerve cord of a lobster "sehr grosse Nerven-Cylinder, welche deutlich hohl sind und Mark führen," and had commented on their gelatinous and transparent nature. A similar account of the corresponding structures in *Astacus fluviatilis* was given by HANNOVER (1842, pp. 90, 91) and by HELMHOLTZ (1842). REMAK almost immediately followed with much more detailed descriptions. He showed (1843, p. 197) that these tubular fibres in *Astacus* measure from one-sixtieth to one-thirtieth of a line (*i.e.*, about 30 to 60 μ) in diameter, and that in the centre of each there is a sinuous bundle of extraordinarily slender fibres, which only occupies one-third or one-fourth the diameter of each tube. Each bundle appears to contain a hundred to several hundred fibres, each about 0·0002 line thick (*i.e.*, about 0·4 μ), an estimate in close agreement with modern results. REMAK suggested that this central bundle is probably equivalent to the axis cylinder of a Vertebrate nerve. His figure (1844, Taf. 12, fig. 8) so closely corresponds to the appearance presented by preparations stained by modern methods to show the neurofibrillæ as to suggest that REMAK actually saw neurofibrillæ in his fresh preparation, which was examined, not in water, but in the animal's blood. REICHERT (1844, p. 194) sought in vain for these central fine fibrils, but they were again seen and clearly figured in a nerve tube of *Astacus* by LEYDIG (1857, p. 60, fig. 33). HAECKEL (1857, pp. 471–478) also gave a careful account of the nerve tubes of *Astacus*, some of which he found attained a diameter of 0·1 mm., while in the œsophageal connectives of a large lobster he observed a single nerve tube, 0·144 mm. in diameter. These memoirs, published previously to 1864 and no doubt well known to LEYDIG, provided him with ample support for his interpretation of the large tubular fibres of Annelids as nervous elements exactly comparable to the broad nerve fibres of Crustacea (LEYDIG, 1864, p. 156).

Lumbricus agricola, forwards to the sub-œsophageal ganglion, where the middle one divided fork-wise and each branch entered the corresponding œsophageal connective and thinned out. The two other fibres, which, just behind the sub-œsophageal ganglion, were connected by a transverse commissure, were also traced as far as the œsophageal connectives (Taf. 4, fig. 8). LEYDIG suggested that the giant fibres, which he stated ran through the whole length of the nerve cord without obvious branches, arose in the brain, for, in *Lumbriculus*, he succeeded in tracing the two branches of the median fibre through the connectives and into the cerebral ganglia.

In the following year LEYDIG (1865, p. 268) referred to the possible presence of a dark-outlined median nerve in *Phreoryctes*, but for fourteen years subsequently no writer, so far as I am aware, labelled the giant fibres as definitely nervous, though during this period they were frequently referred to as "giant tubes," "large tubular fibres" (CLAPARÈDE), "colossal fibres" (KLEINENBERG), "axial canals" (EHLERS), "neural canals" (MCINTOSH), "fibrous band," "tubes" (SEMPER), and "central axis," "canal" and "tube" (VEJDOVSKY). SCHULTZE (1879, p. 106), however, regarded the giant fibre as a nervous element and described its contents in *Lumbricus* as a "central Fibrillenbündel," which, in preparations, readily falls out of the sheath. As he gave no figures of the giant fibre, it is impossible to say whether SCHULTZE actually saw neurofibrillæ or whether the "Fibrillen" were merely the shorter fibril-like artefacts which are often seen in preparations of giant fibres.

From 1880 onwards a more frequent support was given to LEYDIG'S view. LANGERHANS (1880, p. 91) found that the Leydig's fibres in *Prionospio steenstrupi* could be followed through the œsophageal connectives and into the dorsal [cerebral] ganglia, and as they stained black with osmic acid he regarded them as medullated fibres.

A great advance was made by SPENGEL (1881, pp. 37–42), who was able to show that in *Halla parthenopeia* the giant fibres are connected with certain nerve cells remarkable for their size, for they attain a diameter of 0·1 mm. The large nucleus (0·025 mm. in diameter) of these cells is bounded by a thick double-contoured membrane which encloses clear contents in which are strongly staining granules. The cell plasma is finely granular and sends out dorsally a single stout process. Each of the cells has a thick fibrous sheath which is continued along the process. The latter can be traced down the nerve cord as a "neurochord," several of which are seen in every transverse section (eight in the one figured, fig. 53). In each of these neurochords there is a pale indefinitely limited coagulum. Besides these larger neurochords there are several smaller ones seen in section in the fibrous part of the nerve cord. SPENGEL stated that he did not know for certain how many giant cells were present, but that he found about twenty in the first seven or eight ganglia, each of the ganglia having two or three giant cells. Further back the giant cells were absent or very sparse. As the number of large giant fibres was much fewer than that of the cells and appeared to be moderately constant, namely, about seven, SPENGEL suggested that there was a fusion of fibres and that the larger ones

received the smaller ones. He concluded that the "Röhrenfasern" are giant nerve fibres and that they arise from giant nerve cells. He also showed that similar relations exist between the giant cells and fibres in *Arabella*, and he investigated the nature and course of the giant fibres in *Nephthys*, *Lumbricus*, *Lumbriculus*, and *Spirographis*. In *Nephthys* he found two giant cells in the most anterior ganglion of the nerve cord, but in the other three forms just mentioned he sought for them with negative results.

It is evident that HASWELL (1886, p. 756, and Plate 55, fig. 4) observed giant cells, and the giant fibres connected with them, in *Halla australis*, for he describes in the nerve cord of this worm, in the third to the eighth segments, a series of eight or ten oval vesicles, 1/165 inch in diameter, each enclosed in a fibrous capsule, except at one point where a bundle of nerve fibres enters the interior. The capsule is filled with finely granular material and contains a second smaller, more homogeneous, vesicle [the nucleus] about a fourth the size of the larger one, within which is a spherical solid body which stains darkly with hæmatoxylin [the nucleolus]. He was unable to determine the nature of these bodies, but suggested that they might be a rudimentary form of otocyst. They are, however, undoubtedly giant nerve cells.

VIGNAL (1883, pp. 384–386, 403) studied the structure of the giant fibres of the earthworm and showed that staining reactions indicated the presence in the sheath of the fibre of a fatty substance, perhaps analogous to the myelin of vertebrate nerve fibres. He could not, however, see any longitudinal striations in the contents of the giant fibres (differing from SCHULTZE, see above, p. 431). VIGNAL was the first to assign a special nervous function to these fibres: he suggested that they are concerned in insuring the intimate connection of the various parts of the cord, and especially of its two halves, for he noticed that fine tubes passed from the columns of ordinary fibres to open into one or other of the giant fibres.

In 1886 LEYDIG returned to the subject of the giant fibres in order to combat the views of KOWALEVSKY, SEMPER, and VEJDOVSKY (see below, pp. 441, 442), who had all published, since 1864, statements attributing a purely mechanical supporting function to the giant fibres. LEYDIG emphatically reasserted that the giant fibres show a close structural resemblance to the medullated fibres of vertebrates and pointed out that it is not easy to understand how the former can possibly represent a "chorda."

ROHDE (1887) described the general structure of the giant cells of certain Polychætes, their position in the nerve cord, and traced the course of the giant fibres.* He pointed out that, in addition to the anterior giant cells, situated in the

* Besides the giant nerve cells, the course of whose fibres is restricted to the nerve cord, ROHDE described other "giant cells," segmentally arranged in pairs, whose processes cross to the opposite side of the cord and pass out into the spinal nerves. These are probably not homologous with the giant cells described in the text above: they are much more probably to be regarded as motor cells, and will be considered in a subsequent part of this work.

brain and sub-œsophageal ganglion, with processes (giant fibres) directed posteriorly, there are, in some Polychætes, giant cells, the giant fibres of which run forward: for example, in *Sthenelais*, but not in *Sigalion* and *Polynoe*, there is a pair in each segment behind the sixteenth. ROHDE remarked on the presence of fibrils which pass from the axis cylinder in the centre of the giant fibre across the cavity of the latter and into its sheath, and suggested that they pass into the central substance [*i.e.*, the fibrous portion] of the cord, and so the axis cylinder, by means of these fine in- and out-going fibrils, is placed in connection with the central substance. The function of the giant fibres, according to ROHDE, is probably to place in communication widely separated parts of the nervous system.

NANSEN (1887, p. 313) held that there could scarcely be any doubt as to the nervous nature of the giant nerve tubes of Oligochætes. In a second paper in the same year (1887A, pp. 92, 93) he reaffirmed his belief in their nervous function, based on an examination of these tubes in *Lumbricus*, and stated that their contents are entirely composed of numerous closely apposed minute "primitive tubes."

FRIEDLÄNDER (1888) examined the giant nerve fibres of *Lumbricus* and demonstrated, for the first time in Oligochætes, the connection of these fibres with cells in the nerve cord. He showed that the two lateral giant fibres arise as processes from cells of special structure, but not "giant" in size, situated a short distance from the posterior end of the nerve cord, thus definitely proving the homology of these fibres in Oligochætes with the giant fibres of Polychætes. He also showed that in their further course forwards each of these lateral giant fibres receives processes from other ganglion cells of essentially similar nature to those from which the fibres take their origin. Some (p. 73 and Taf. 10, fig. 6) or many (1894A, p. 668) of these cells are stated to be bipolar, the process from one pole passing outwards into the giant fibre and that from the other pole inwards, but its subsequent course was not traced. No other bipolar giant cells have, so far as I know, been found in Annelids.

HALLER (1889) described in each segment of *Lepidasthenia elegans* a pair of "giant cells,"* the processes of which cross the nerve cord and pass into the spinal nerves. While admitting the origin of these smaller "giant fibres"* from corresponding "giant cells," HALLER attributed the formation of the large giant fibres† to the enclosure of some of the ordinary fibres of the "central nervous network" [*i.e.*, the fibrous portion of the nerve cord] in a common sheath. He found that the giant fibres of *Lumbricus* were so closely associated with the "central network" that they might also be considered to be partly derived from this "network." He concluded that the giant fibres are functional nerve fibres.

FRIEDLÄNDER (1891), as the result of a long series of observations, concluded that,

* These cells are not homologous with the giant cells of *Halla*; they are almost certainly large motor cells, and their processes are consequently not comparable to the giant fibres of *Halla*.

† These are true giant fibres.

especially in *Mastobranchus* and *Lumbricus*, the sheath of the giant fibre consists, at any rate in great part, of a myelinogenous substance nearly allied to that of the medullary sheath of vertebrate nerve fibres. He was, however, unable to distinguish any definite structure in the contents of the giant fibre. He showed that the giant fibres of *Mastobranchus* are outgrowths of large ganglion cells, to which conclusion Eisig (1887, p. 460) had also arrived in his work on the same animal, though the latter observer had not been able to actually demonstrate the connection between the giant cell and its fibre. Although Friedländer was unable to find any conducting elements (fibrils) in the thin watery contents of the giant fibres, he still argued (using a *reductio ad absurdum*) that they must be nervous, for the nerve cords of *Palæmon* are composed of tubular nerves similar to, but smaller than, the giant fibres; if the former are not nervous, then the nerve cord of this animal would be composed chiefly of non-nervous elements. The giant fibres are therefore to be regarded as medullated nerve fibres.* Friedländer briefly outlined in this paper his view regarding the function of the giant fibres, namely, that they are correlated with the almost simultaneous contraction of all the segments of the worm. This interpretation of their function was further discussed in his subsequent papers (1894, 1895). In the meantime Cerfontaine (1892) discovered that, in *Lumbricus*, the median giant fibre arises as the result of fusion of processes of several specialised cells situated in the anterior part of the nerve cord, and he confirmed Friedländer's observations as to the similar origin of the lateral giant fibres from special cells situated in the posterior ganglia. Although Cerfontaine succeeded in staining the contents of portions of the giant fibres with methylene blue, it is worthy of note that this method failed (as it has invariably done in Annelids) to demonstrate the connection of the giant fibre with its giant cell; this connection was proved by means of serial sections. Cerfontaine also showed that the giant fibres give off branches moderately regularly along their course, which either subdivide and end in the nerve cord or "se dirigent vers les troncs nerveux et vont vers la périphérie comme fibres motrices." There are frequent anastomoses between the branches which arise from the lateral fibres. Cerfontaine agreed with Friedländer's view of the function of the giant fibres, to which support was also given by Joest (1897) and Korschelt (1898) as a result of their experimental work on earthworms.

Wawrzik (1892) described the sheath of the neural canals of several Chætopoda, and stated that this sheath is a continuation of the subcuticular fibrous tissue which envelops the giant ganglion cells from which the canals take their origin. But not only does this tissue envelop the nerve elements, it also penetrates into their interior, becoming continuous with the spongioplasmic fibrillæ of the "neural canal." Wawrzik considers that his observations establish the histological identity of the spongioplasm and the fibres of the sheath, and consequently that the fibrillæ of the axis cylinder of the neural canal and the corresponding "mitom" of the ganglion cell

* See also Friedländer, 1894, 1894a, 1895.

are to be regarded only as a supporting substance (Stützgerüst), and not as essentially nervous, the nervous function being, presumably, fulfilled by the homogeneous liquid hyaloplasm.

Although it is not my intention to give an account of the giant fibres in Crustacea, the work of ALLEN (1894, pp. 466, 467) deserves special notice in this connection. He was able to show, by means of methylene blue preparations, that in the embryonic lobster there are on the ventral surface of the brain two (a pair) large cells, from each of which a moderately thick fibre arises, crosses to the opposite side, and leaves the brain by the œsophageal connective. On entering the first thoracic ganglion these two fibres become very broad, having a diameter many times greater than that of any other fibre in the body; they are, in fact, the giant fibres. They run the whole length of the nerve cord to the last (sixth abdominal) ganglion, in which they divide into several branches.* These giant fibres give off a few branches to the neuropile of the brain, especially at the angles of decussation, but there are no collateral branches along their course down the nerve cord; the fibres end, as already stated, in branches in the last ganglion. As ALLEN pointed out, they serve the purpose of putting some organ at the posterior end of the abdomen into direct connection with the brain.

APÁTHY'S classical memoir (1897) contains important information on the subject of giant fibres. The neurochords of *Lumbricus* are described as containing thin (with sometimes one or two thicker) wavy neurofibrillæ, some of which here and there are seen issuing through the glia sheath into the fibrous mass of the cord. The neurochords are homologised with the "sensorische Schläuche" of *Hirudo*, but no reference is made to the fact that FRIEDLÄNDER and CERFONTAINE had traced the giant fibres in *Lumbricus* into nerve cells, nor does APÁTHY give any indication of the mode of origin of the "neurochords" of *Lumbricus* or of the "sensorische Schläuche" of *Hirudo*, to which he compares them.

LEWIS (1898) investigated the nervous system of two Maldanids, *Axiothea torquata* and *Clymene producta*, and concluded that the "Leydig's fibres" therein present agree in structure with those described by FRIEDLÄNDER. The contents of "Leydig's fibre" are uniform throughout, so that it represents a single nerve fibre and not a bundle of fibres. In *Clymene* two "Leydig's fibres" apparently arise, in the subœsophageal ganglion, from a pair of giant cells, and soon fuse to form a single median fibre which receives the processes of the remaining giant cells, but nevertheless increases only very slightly in diameter. In *Axiothea*, from the seventh to the nineteenth segment, there are two "Leydig's fibres," otherwise the arrangements are as in *Clymene*. No branching of these fibres was seen in either worm, and none was believed to exist. The giant cells are situated in the nerve cord without any discoverable regularity or symmetry. Each cell has an excentric nucleus and a

* RETZIUS (1890, p. 35) states that in *Astacus* the branches of the giant fibres in the sixth abdominal ganglion pass out through the posterior nerve twigs, in which they further divide.

centrosome and sphere. Miss LEWIS concluded that "Leydig's fibres" are true nerve fibres, and do not, in these animals, function in any way as an organ of support.

HAMAKER (1898), who studied the nervous system of *Nereis virens*, showed that of the three giant fibres which traverse the entire length of the nerve cord of this worm, the two larger lateral ones break up in the œsophageal connectives, into branches which apparently pass to the optic ganglion of the brain, while the smaller median one terminates in the subœsophageal ganglion in branches, one of which is in connection with a cell in this ganglion. All the giant fibres extend posteriorly into the last body segment without branching, but they are pierced by many smaller fibres (HAMAKER'S "Set B") which pass directly through them, and by means of which the giant fibres are put into relation with every segment of the body. The giant fibres were not traced to giant cells; indeed, no giant cells are described. Nervous relation between the giant fibres and the other fibres is, according to HAMAKER, established directly between the respective axis cylinders. He believes, with FRIEDLÄNDER, that the giant fibres are associated with the sudden longitudinal contraction of the worm, which, in the case of *Nereis virens*, took place when there was apparently no stimulus except the passing of a shadow.

Giant cells and fibres occur in all the species of *Arenicola* investigated by GAMBLE and ASHWORTH (1900) except *A. claparedii*, but they were specially examined in *A. grubii*. The giant cells in this animal occur in couples, in the mid-ventral part of the nerve cord, close to the hinder border of each segment. Each cell is from 50 μ to 80 μ in diameter and is surrounded by a fibrillated glia sheath from which fibril-like processes pass into the outer layers of the cytoplasm. In some of the preparations indications of neurofibrillæ were seen in the cytoplasm and traced a short distance into the stout process of the cell—the giant fibre. This process, after giving off a branch whose ramifications extend into both halves of the fibrous part of the nerve cord, enters the median, or, more generally, one of the lateral giant fibres. The three giant fibres were found to be frequently connected with each other, especially in the chætigerous annuli, by means of anastomoses. While the giant cells are apparently not differentiated in a post-larval specimen 4·5 mm. long, in specimens 17·5 mm. and upwards in length they are well marked and they are no larger in examples 250 mm. long than in others only one-fourth this length (ASHWORTH, 1904, pp. 49–51). The giant cells in *Arenicola assimilis* (ASHWORTH, 1903) have also a segmental arrangement, there being one or two in each of the body segments.

RABES (1902) denies that the giant fibres fulfil the function ascribed to them by FRIEDLÄNDER. His objections were founded solely on the ground that in earthworms in which the nerve cord had been severed and time given for regeneration, a stimulus was transmitted through the scar tissue although no visible union of Leydig's fibres could be demonstrated. His observations, while convincing him that these fibres are not concerned in causing the spasmodic contraction, do not,

however, permit him to draw a definite conclusion for or against their nervous nature.

SCHNEIDER (1902, p. 403) confirms APÁTHY'S description of the contents of the giant fibre. He finds a bundle of very fine fibrillæ, with one or more somewhat thicker ones, in the giant fibres of *Eisenia* (*Lumbricus*) *rosea* and states that from this bundle fibrils pass out, by way of the branches of the giant fibres or directly through the myelin sheath, into the neuropile.

RAMON Y CAJAL (1904) briefly describes the appearance of the three giant tubes of *Lumbricus*, as seen in preparations made by his new method, but he was unable to ascertain their place of origin. A single neurofibril* runs, in a spiral course, in each tube; the central tube, although the largest, contains the most delicate fibril. He is unable to confirm APÁTHY'S statement that there are several fibrillæ in each tube, some coarse and others fine. He states that the fibrillæ in the giant tubes are much thinner than those of the majority of the commissural and motor axones, a fact which probably explains why those who have investigated these tubes by means of the methods of GOLGI and EHRLICH have doubted their nervous nature.

KRAWANY'S recent researches (1905) on the nervous system of the earthworm do not add much to our knowledge of the giant fibres. He states that in many places a fibril is very clearly differentiated from the more feebly stained envelope, that anastomoses between the giant fibres occur in each ganglion, and that branches are given off. His fig. 6 (Taf. 3) shows a single moderately thick fibril in the giant fibre. No reference is made, either by SCHNEIDER, CAJAL, or KRAWANY, to the cells from which the giant fibres arise.

EISIG (1906) described in the nerve cord of *Ichthyotomus* cells of relatively large size and also large transparent fibres, but it is not apparent from the description and figures that there is any connection between these cells and fibres. Giant sensory "Markfasern" enter the sensory part of the ganglia of the nerve cord and form a branching "basket work" around the cells. These fibres may be followed from the receptors of the ventral cirri† to their central ending in the cord, so that they are of sensory nature. Similar "Markfasern" arise in clusters in each segment from the sensory part of the ganglia and end in the integument in "segmental ventral organs." There are also longitudinally running medullated fibres in the connectives of the nerve cord, but it is not clear that these are homologous with ordinary giant fibres, as there is no evidence that they run continuously through any considerable distance. EISIG agrees with FRIEDLÄNDER that the giant fibres of Annelids serve for the transmission of the impulse which produces sudden contraction, and concludes that not only are the giant fibres connected with the motor nerves of

* The giant nerve fibres of the earthworm figured by MICHEL (1899, Pl. 26, figs. 10, 11) contain a single undulating fibril, and those by JOSEPH (1902, fig. 26) one, or at most two, fibrils.

† Similar tubular fibres with well-marked contours are present in the cirri of *Hermadion fragile* (CLAPARÈDE, 1868, pp. 75, 76, Pl. 5, fig. 2 F).

the longitudinal muscles whose contraction causes the sudden movement, but are also connected with the podial receptors, which are present in each segment, presumably by the above described giant sensory "Markfasern."

Besides those above mentioned, who have described the giant fibres in detail, there are many other writers who have briefly referred to them as elements of the nervous system.

(a) *In Polychætes.*—MEYER (1882, p. 788) recorded the presence of giant fibres in *Polyophthalmus* and traced the course of the large giant fibres in *Myxicola* (1888, p. 559). JACOBI (1883, p. 24) stated that the single "Röhrenfaser" of *Polydora* is filled with a nerve-like substance. PRUVOT (1885, p. 239) described the canals, filled with a hyaline substance, in *Nephthys* and other Polychætes (pp. 264, 271, 315), but could not trace their connection with cells. BRUNOTTE (1888, p. 32) traced the well developed giant fibres in *Branchiomma* into the brain, where he found that they divide into numerous small branches. TREADWELL (1891, p. 278) traced the course of the tubular fibres in *Serpula*. DE SAINT-JOSEPH (1894) recorded under various names ("fibre tubulaire," "fibre tubulaire colossale," "fibre nerveuse colossale") the presence of giant fibres in many Polychætes and stated that in *Bispira volutacornis* the tubes contain an orange-coloured liquid and in *Lanice conchilega* a brownish liquid, and that those of *Sabella* arise in the brain. FAUVEL (1897, p. 354) demonstrated in some of the Ampharetidæ, *e.g.*, *Ampharete grubei*, the connection of certain neural canals in the ventral nerve cord with giant cells, but as he states that these canals are present only in the first six or eight thoracic segments and are not continuous but run only for a distance of one or two segments, it is doubtful whether they are true giant fibres. FAUVEL declined to subscribe to the view that these elements in Polychætes are supporting structures. ATTEMS (1903, pp. 185, 186) described, in *Scololepis fuliginosa*, giant cells which are situated two here and there in the nerve cord near the neurochord, but he could not find any connection between the cells and the neurochord or between the latter and the other elements of the nerve cord. ALLEN (1904, p. 105) found two fibres of large size in the nerve cord of *Pœcilochætus*, but their connection with ganglion cells was not traced.

(b) *In Oligochætes.*—PERRIER (1874, pp. 363, 364, 510) and BENHAM (1886, p. 290) have noted the presence of giant fibres in several Oligochætes. NASSE (1882) recorded the presence of "Röhrenfasern" in Tubificidæ, and stated that no branches of them were observed. KULAGIN (1890, p. 404) found that the contents of the "nerve canals" of certain Russian Oligochætes consist of fibres embedded in a protoplasmic mass. ABEL (1903, pp. 55, 57) recorded the regeneration of the giant fibres in *Nais* and *Tubifex*.

(c) *In Echiuroidea.*—A structure corresponding to a giant fibre appears to be also present in *Echiurus*. GREEF (1872, p. 106) first described this structure in *E. pallasii** as a central canal running through the whole length of the nerve cord

* For figures, see GREEF, 1879, figs. 20 A, 21, 22.

and passing into the œsophageal connectives.* SPENGEL (1880, pp. 486, 487) pointed out that the canal, which is dorsal and not central, resembled the giant fibres of Polychætes, with which he believed it to be homologous. Miss EMBLETON (1900, p. 86), who saw the corresponding canal in the nerve cord of *E. unicinctus*, also considered it to be a giant fibre, although she was not able to satisfactorily trace its connection with ganglion cells. SEITZ (1907) has recently described a neural canal in *Urechis* (*Echiurus*) *chilensis*, but states that it appears to have no special envelope, but is rather like a tubular space in the lower part of the dorsal connective tissue of the cord. Its gelatinous contents are sometimes divided into two closely applied bands of unequal thickness; in other places the band forks, the weaker branch runs ventrally but cannot be followed. The termination of the canal could not be ascertained, nor could any connection between it and ganglion cells be demonstrated. SEITZ does not express any opinion as to whether it is of nervous nature.

(d) *In Abnormal Annelids.*—ANDREWS (1894, p. 438) found that the giant fibres were present in both portions of a posteriorly branched specimen of *Allolobophora fœtida*. PRENTISS (1901) described a specimen of *Nereis virens* which presented duplication of certain parts, and in which a supernumerary nerve cord extending through fifteen segments was present. He found that the three giant fibres, which form such conspicuous features in the normal cord, were absent in the supernumerary cord (p. 569). The latter nerve cord probably developed quite independently of the normal cord (p. 571).

II. The Giant Fibres regarded as Degenerate Nerve Fibres.

EISIG (1887), chiefly as a result of his study of the giant fibres in *Mastobranchus*, concluded that, although these "neurochords" are almost certainly the processes of giant ganglion cells (see above, p. 434), and that they are originally nervous elements, the nerve fibres which they contain gradually degenerate. He regarded certain pale, broad, spiral structures, which he saw in these neurochords, as the degenerating nerve fibres. The wall or sheath of the neurochord subsequently becomes thicker; all trace of the spiral fibres, in the lumen of the neurochord, disappears, and in their place a watery fluid is found. EISIG considered that the resultant neurochord serves as a supporting organ which may be compared functionally, but not genetically, to the chorda.

LANG (1894, p. 221) and PERRIER (1897, p. 1596) adopted EISIG'S views regarding the nature and function of the giant fibres.

It is shown in the present memoir that the giant fibres of *Halla* are true nerve fibres which issue from giant nerve cells, and that these fibres contain typical neurofibrillæ which are in connection with an elaborate fibrillar network in the giant cells. When examined in the living or fresh condition, the contents of the giant fibres of

* A similar canal is figured by GREEF in the nerve cord of *Bonellia viridis* (1879, Taf. 23, fig. 87), but VEJDOVSKY could not convince himself of its presence (1882, p. 54).

Halla, like those of the giant fibres investigated by EISIG, have a semi-fluid, or, in some cases, almost watery, consistency, but their fibrillar elements are well developed and exhibit no sign of degeneration. Similar neurofibrillæ are present in the giant fibres of the earthworm, and, on future investigation, will probably be found in all the giant fibres of Annelids. Observations on the giant fibres of *Halla* and the earthworm are directly opposed to the view that the nerve elements in these fibres have undergone degeneration. The structures which EISIG observed in the giant fibres and regarded as degenerating nerve fibres are, as shown by FRIEDLÄNDER (1891, p. 210, *et seq.*), folds and processes of the sheath of the giant fibre which are especially obvious when the latter is contracted, but disappear when the giant fibre is stretched.

III. The Giant Fibres regarded as Non-nervous Elements on account of their Staining Reactions.

The denial of the nervous nature of the giant fibres has depended, in at least three cases, upon the fact that the methods usually adopted for staining ordinary nerve cells and fibres failed to stain these peculiar fibres.

According to SEMPER (1876, p. 202, footnote) Leydig's fibres are not true nerve fibres, as is apparent by their reaction to stains; those of *Nais** do not stain with gold chloride and stain with carmine only with difficulty. SEMPER regarded these tubes as containing an easily coagulable substance, which is not blood, because the coagulated fluid reacts to stains quite differently to blood plasma and cells are never seen in it.

VON LENHOSSÉK (1892, p. 121) stated that the giant fibres of *Lumbricus** never blackened when treated by GOLGI's method; they remained unstained even when the ordinary nerve fibres were quite black, and, moreover, they were surrounded by an unbroken sheath.

RETZIUS (1892, p. 15), after investigating the giant fibres of *Lumbricus*,* agreed with VON LENHOSSÉK that these elements could scarcely be considered to be nerve fibres because: (1) they resist staining by GOLGI's method; (2) they never branch; and (3) they do not arise from cells.

The particular staining reaction upon which such great dependence for specific staining and diagnosis of nerve elements was placed is admittedly very capricious; there are many undoubted nervous structures in almost any Golgi preparation which have not been blackened by this process, and therefore it cannot be relied upon as a test for nerve elements. As a matter of fact, APÁTHY and others have since shown that these very structures investigated by VON LENHOSSÉK and RETZIUS, namely, the giant fibres of *Lumbricus*, contain typical neurofibrillæ and are certainly nervous in spite of their non-impregnation by the method of GOLGI. The giant fibres

* It is worthy of note that the majority of those who have denied the nervous nature of the giant fibres have confined their attention to the examination of these structures in Oligochætes.

of *Halla* also resist staining by this method (see p. 475), but are nevertheless true nerve fibres (see pp. 483–488).

The second objection of RETZIUS to the nervous nature of the giant fibres, namely, that they do not give off branches, is astonishing when one considers that branches of the giant fibres of the earthworm had been previously described by VIGNAL (1883), HALLER (1889), and FRIEDLÄNDER (1888, 1891), and branches of the giant fibres of certain Polychætes by ROHDE (1887). This objection became quite untenable in the light of the observations immediately published by CERFONTAINE,* FRIEDLÄNDER, and others.

RETZIUS' third statement—that the giant fibres do not arise from cells—is also remarkable when it is borne in mind that eleven years previously SPENGEL (1881) had demonstrated the origin of the giant fibres of *Halla* from giant cells, that ROHDE (1887) had found a corresponding relationship between the giant cells and fibres of certain other Polychætes, and that FRIEDLÄNDER (1888) had shown that the lateral giant fibres of *Lumbricus* arise from cells. These observations, and others published by CERFONTAINE (1892),* effectually dispose of RETZIUS' third objection to the nervous nature of the giant fibres.

IV. The Giant Fibres regarded as Supporting Organs homologous with the Notochord of Vertebrates.

KOWALEVSKY was the first to compare the giant fibres of Annelids to the notochord of Vertebrates. The institution of this comparison was no doubt largely influenced by the theory of the Chætopod ancestry of Vertebrates. Those who advocated this view surmounted the difficulty of the different positions of the nerve cord in Annelids and Vertebrates by adopting DE BLAINVILLE'S conception, that the dorsal and ventral surfaces of Vertebrates are reversed as compared with those of Annelids. Consequently when figures of transverse sections of a Vertebrate and an Annelid were placed side by side for comparison, the section of the worm was drawn inverted, *i.e.*, the ventral surface was placed uppermost, in order to bring the nerve cords in the two figures to the upper side.

KOWALEVSKY (1871, p. 20), in describing the development of *Euaxes* (= *Rhynchelmis*), referred to three large cells which lie below (as the figure is inverted, that is, dorsal to) the ganglion. He believed these to be associated with the structures which he took for Leydig's fibres. As these cells are mesoblastic and lie outside the "inner neurilemma," he pronounced against the nervous nature of the fibres, which he suggested were stiff cords comparable functionally and genetically† to the chorda dorsalis of Vertebrates.

SEMPER (1874, pp. 50, 51) compared the structures seen in a transverse section of

* See above, p. 434.

† BÜLOW (1883, p. 92) pointed out that the neurochords could not be homologised with the chorda dorsalis of Vertebrates, as the former were mesoblastic and the latter hypoblastic in origin.

a Vertebrate and of an Annelid (the latter inverted) and concluded that the giant fibres correspond to the chorda dorsalis in position and relations. Later (1876, p. 202), however, he withdrew this interpretation,* for he had then discovered, in the growing tail of *Nais*, the mesoblastic "chorda cells," which he regarded as forming a sort of axis corresponding in position and function to the chorda of Vertebrates (pp. 168, 169). These "chorda cells" have, of course, no connection whatever with the giant fibres (see RANDOLPH, 1892).

CUNNINGHAM (1885, p. 12) "got back . . . . to the old idea of the homology of the notochord and the three giant fibres beneath† the nerve cord in the earthworm," and PERRIER (1881, pp. 228, 229) remarked that the three longitudinal cords of *Pontodrilus* recall, by their position, the chorda of Vertebrates.

The connection of the giant fibres with undoubted nerve cells and the fact that they contain typical neurofibrillæ are sufficient to dispose of the idea that these fibres have any homology whatever with the notochord of Vertebrates.

V. The Giant Fibres regarded as Supporting Organs, but not homologous with the Notochord of Vertebrates.

VEJDOVSKY regarded the giant fibre in *Rhynchelmis* (1876, p. 340) as a hollow central axis‡ separating the two halves of the nerve cord, and that of *Tomopteris* (1878, p. 88) as a medullary canal representing the remains of a groove of invagination of the ectoderm, but in 1879 he pronounced the giant fibres of *Enchytræus* to be tubes of a cartilaginous substance, but did not adduce any histological evidence to support this statement. In his 'System und Morphologie der Oligochaeten' (1884) he brought forward the view, to the support of which he contributed further arguments subsequently, that the neurochord§ forms a peculiar "Accommodationsapparat" which serves to support the ventral nerve cord during the bending and contraction of the body, an interpretation which he regarded as highly probable because the neurochord is best developed in those forms in which the muscles of the body wall are weak (*e.g.*, *Lumbriculus*, *Rhynchelmis*), while it is absent in *Phreoryctes*, which is remarkable for the strength of its body wall. VEJDOVSKY also stated that the neurochord arises, in ontogeny, simultaneously with the muscles and from the same mesoblastic elements as the longitudinal muscle

* SEMPER'S view of the nature of the giant fibres, as expressed in 1876, has been already given, see above, p. 440.

† The worm being inverted.

‡ A similar view had been previously put forward by RATZEL (1868, p. 577), who considered the corresponding structure in *Lumbriculus* to be formed as a consequence of the development of the nerve cord in two halves, which later united in the middle line.

§ The term neurochord, as used by VEJDOVSKY, includes the connective tissue band and its contained tubes (giant fibres), which in many Oligochætes form a compact mass in the mid-dorsal region of the nerve cord.

bands, the neurochord being developed from a median row of mesoblastic cells,* and the longitudinal muscles from lateral groups of similar cells. The neurochord is at first a solid connective tissue band in which the tubes arise later. He regarded SPENGEL'S view (for which see above, p. 431) of the origin and nature of the giant fibres as quite untenable (p. 87).

In 1888 (pp. 199, 200) VEJDOVSKY described how the neurochord tubes (= giant fibres) of a living specimen of *Rhynchelmis* become twisted in a screw-like manner in the contracted condition of the animal, and he held that no one who saw them act in this way would ever take them for giant fibres.†

In 1892 (p. 383) VEJDOVSKY no longer ascribed the origin of the series of neurochord cells* to the mesoblast (see above), but stated that they have the same origin as the neuroglia cells. He held that the neurochord Anlagen in each ganglion are originally separate and only secondarily fuse and that the neurochords are at first solid, but later processes of ganglion cells run, as a bundle of nerves, through the median neurochord;‡ the lateral ones are solid for some time longer, but are eventually also provided with a nerve bundle. He clearly stated (p. 385) that there grow, into the original substance of the neurochords, neuroglial tubes, into and through which the processes of ganglion cells pass and thus form the bundle of nerve fibres of the neurochord.‡

In his famous work on the embryology of the earthworm, WILSON (1889, p. 418) described the appearance of the neurochords in the upper part of the fibrillar mass of the nerve cord and stated that, even in the earliest recognisable condition, the three characteristic fibres were already present.§ His preparations showed that the giant fibres unquestionably arise in the fibrillar portion of the nerve cord and not from the mesoblastic investment, from which they are separated by a distinct line. "There can be little doubt . . . . of the essential correctness of the original view of LEYDIG and CLAPARÈDE,‖ according to which the colossal fibres of the neurochord

* BEDDARD (1895, p. 23) accepted VEJDOVSKY'S account of the development of the neurochord tubes from a row of large cells (*cf.* the observations of WILSON and VON WAGNER, cited below), but at the same time expressed his belief in the nervous nature of these tubes.

† FRIEDLÄNDER (1891, p. 209, footnote) pointed out that the contraction of the body wall would necessitate the coiling or folding of the less contractile neurochord, but he could not believe that the windings of the latter were as regularly screw-like as described by VEJDOVSKY.

‡ This interpretation of the relationship of the nervous substance and the sheath of the neurochord or giant fibre is equivalent, as FRIEDLÄNDER (1891, p. 209, footnote) remarked, to stating that the axis cylinder of a vertebrate nerve serves to innervate the sheath!

§ This statement, while at variance with VEJDOVSKY'S account, agrees perfectly with the observations of VON WAGNER (1905, p. 110), who states that in the regenerating nerve cord of *Lumbriculus* the neurochord appears quite suddenly in the correct position and with the correct number of tubes, and that it is almost the last structure to be formed in the regenerated nervous system.

‖ CLAPARÈDE may possibly have entertained such a view, though I do not recall any specific statement of his attributing to the giant fibres a supporting function. LEYDIG staunchly upheld his original view of

are specially modified nerve fibres which have probably assumed a supporting function."

Bülow (1883, p. 92) concluded that the neurochords in *Lumbriculus* are not of nervous nature, but are elastic structures, not, however, homologous with the chorda of Vertebrates (see above, p. 441, footnote).

Michaelsen (1886, p. 33) observed muscle fibrils running along the dorsal wall of the single large neurochord in *Pachydrilus beumeri*, and argued from this fact that the neurochord functions as a supporting organ.

Eisig (1887) regarded the neurochord, after the degeneration of the nerve fibres which it originally contained, as a supporting structure, a view adopted by Lang (1894) and Perrier (1897) (see above, p. 439).

Jourdan (1887, p. 262) concluded that there were no nervous structures in the giant fibre of *Eunice*, and, consequently, he could not regard it as a nerve tube, but considered it to be a supporting structure.

Cunningham (1888, p. 275) failed to establish any connection between the ganglion cells and neural canals of certain Polychætes. He regarded the canals as supporting elements which serve to prevent the nerve cord being bent at a sharp angle: the cord thus escapes injury during the wriggling of the worm. He stated that the canals attained their greatest development in worms which are very long in proportion to their thickness, and in those in which the nerve cord, owing to its non-separation from the epidermis, is more liable to injury. Fauvel (1897, p. 354) denied the validity of this argument, and instanced the case of *Ampharete*, in which the canals are developed only in the thorax, where the nerve chain is completely separated from the integument, and where it is protected by the thick ventral "bucklers," while in the slender abdomen, which is more liable to quick movements and great folding and where the nerve cord is epidermal, the canals are absent.

According to Garman (1888) the neurochords of the earthworm *Diplocardia* consist principally of neurilemma, and they subserve a supporting function.

Spencer (1888, p. 20) pointed out that the giant fibres of *Megascolides* are remarkable for the very definite central rod of homogeneous gelatinous material, and for their equally definite enclosing sheath of connective tissue. No connection was found to exist between these fibres and any of the nervous elements, and the "usually accepted idea"—that the giant fibres have solely a supporting function—appeared to Spencer to be probably the correct one. He agreed with Cunningham that they may also serve to prevent the nerve cord being bent at a sharp angle, and he considered that Vejdovsky's term, neurochord, was the most applicable to these structures.

The connection of the giant fibres with undoubted nerve cells and their possession of typical neurofibrillæ prove that these fibres are essentially nervous, and not

their truly nervous nature and, especially in his paper published in 1886, denied the mechanical *rôle* attributed to the giant fibres by some other writers.

merely supporting, elements. The thickness of the sheath of the giant fibre, which in many cases is considerable, has probably afforded the chief reason for regarding these fibres as supporting structures of the nerve cord. But the strength of the sheath may be accounted for in another way. The axis cylinder of the giant fibre is of an unusually soft and yielding nature, being described as almost watery in some cases, and therefore is in special need of efficient protection from undue compression and from bending at sharp angles during the contraction of the worm. The stoutness of the sheath may be regarded as correlated with the nature of the substance—the essential element of the giant fibre, its axis cylinder—which it encloses and protects.

VI. The Giant Fibres regarded as Canals with no Definite Function.

Claparède (1869, pp. 588–590) described Leydig's view of the nervous nature of the giant tubes as very seductive, but as having little support from the course and relations of the tubes, for while these latter run from one end of the nerve cord to the other they do not enter into organic connection with the nervous elements. He regarded the three giant fibres of *Lumbricus* as being completely isolated from the true nervous system and as having no connection even with each other, for he was unable to find the commissure described by Leydig. He recorded (1868 and 1871) the presence of large tubular fibres in the nerve cord of ten genera of Polychætes. In 1873 he again stated that he regarded the tubular fibres (to whose enormous size in the Serpulidæ he draws attention) as completely exterior to the nerve cord proper, although he found them, for instance, in *Spirographis*, extending into the brain and there dividing into several secondary branches. Claparède considered it superfluous to formulate hypotheses regarding the function of these fibres (p. 122).

Ehlers (1864—1868) recorded the presence of "axial canals" in four genera of nereidiform Polychæta, and McIntosh (1878 ; 1878a, pp. 453, 455) published a long list of Polychæta in which "neural canals" are present.

Schröder (1886, p. 29) described the three large neural canals of *Nereis* and stated that their significance was unknown. The presence of neural canals is mentioned by Steen (1883, p. 237) in *Terebellides strœmii*, by Collin (1888, p. 483) in *Criodrilus lacuum*, and Izuka (1905, p. 247) records in *Ceratocephale* the presence of two neural tubes.

VII. The Giant Fibres regarded as Nutritive Tubes.

According to Emery (1886, p. 397), the tubular colossal fibres in the nerve cord of *Nephthys scolopendroides* have absolutely nothing in common with nervous elements, but are associated with the nutrition of the nervous system, thus resembling lymphatic vessels. When first formed they are filled, like other cavities of the body, by a "transudat," and there are also in the canals certain nucleated cells which perhaps serve to secrete the liquid contents.

SCHACK (1886, pp. 31, 32) described the canals in *Nephthys cæca* and expressed the opinion that they are not tubes with walls of their own but lysigenous canals, *i.e.*, intercellular spaces.

MICHEL'S view (1898, pp. 345, 346) of the nature of these structures is similar to that of EMERY. He described, in regenerating buds of *Allolobophora*, irregular and anastomosed spaces among the nervous elements which later become regular and form longitudinal canals, which, however, remain for a long time feebly outlined. In adult specimens of *Allolobophora* there is to be seen, at certain points of the canal, an undulating filament, which MICHEL regarded as having "strayed" thither. After studying also the giant fibres of *Nereis*, *Nephthys*, and especially *Nerine*, he came to the conclusion that it is difficult to avoid the impression that they are sinuses with liquid contents.

In a later paper, MICHEL (1899, p. 486) still seems to hold to the opinion that the giant tubes (of *Lumbricus*) are lacunæ into which certain nervous fibrils have "gone astray," the liquid contents of the tubes being possibly secreted by the giant cells.

This view of the nature and function of the giant fibres—that they are nutritive tubes—is no doubt largely due to the semi-fluid nature of their axis cylinders, but is, of course, rendered untenable by the proof that these fibres are connected with nerve cells and that they contain neurofibrillæ.

VIII. The Giant Fibres regarded as Blood-vessels.

OWSJANNIKOW (1900, pp. 23, 25) declares that the so-called giant fibres of the Crayfish are really blood-vessels. He bases this interpretation on three grounds: (1) their walls are thicker than the sheaths of the nerve fibres; (2) he has found blood corpuscles in sections of the giant fibres; and (3) in methylene blue preparations of the giant fibres he has clearly seen endothelium and muscle and sometimes blood corpuscles.

The first of these reasons for regarding the giant fibres as blood-vessels is of no weight whatever, for it is now well known that in many cases the thickness of the sheath of the various nerves of the same animal is subject to great variation. A fundamental objection to the interpretation advanced by OWSJANNIKOW is afforded by ALLEN'S observations, of which OWSJANNIKOW takes no note, that the giant fibres of the lobster, which are, of course, homologous with those of *Astacus*, definitely arise from large cells in the brain (see p. 435).

3. THE GIANT NERVE CELLS AND GIANT NERVE FIBRES OF *HALLA PARTHENOPEIA*.

The following observations are based upon an examination of more than fifty specimens of *Halla parthenopeia*, the smallest of which was 1·4 cm. in length (when preserved), and the largest 110 cm. (when living and fully extended).

There are two series of giant nerve cells present in *Halla*, an anterior series found only in the first few ganglia, and a posterior series in the last few ganglia of the ventral nerve cord. In the present account the anterior series only is described in detail, the posterior giant cells are briefly noticed on p. 488. Unless otherwise expressly stated, the giant cells referred to in the subsequent pages of this paper are those of the anterior series.

I. The Number and Arrangement of the Giant Cells.

The number and arrangement of the giant cells of *Halla* exhibit considerable variation according to the age and size of the specimen, but an examination of a long series of worms of various sizes renders it clearly evident that there is primarily a couple of giant cells in each of the first few chætigerous segments, though, occasionally,* only one cell is found instead of a couple. The two cells of each couple are generally found close together,† one behind the other, in or near the mid-ventral region of the nerve cord, and are usually so placed that the hinder cell lies near the posterior border‡ of the segment to which the couple belongs (fig. 52).

These remarkable cells are generally easily recognised, in sections of the nerve cord, by their large size and the thick sheath with which each is enveloped (fig. 52); but in several specimens, in the segment behind the one containing the last undoubted couple of giant cells, there are two cells which occupy positions corresponding to those of the giant cells of other segments, but which are still comparatively small (30 to 40 μ in diameter) and have only thin sheaths. It is sometimes difficult to determine whether these are giant cells or large ganglion cells, but they are in most cases to be regarded as giant cells which have only recently been differentiated.

It has, unfortunately, not been possible to obtain very young specimens of *Halla*. The youngest one in my possession, although only 14 mm.§ in length, has already ninety segments, and a couple of giant cells is present in each of the first five chætigerous segments (see fig. A). In another specimen, which possesses 126 segments and is 44 mm.§ in length, there is also a couple of giant cells in each of the first five segments (see fig. B). Examination of a specimen with 237 segments,

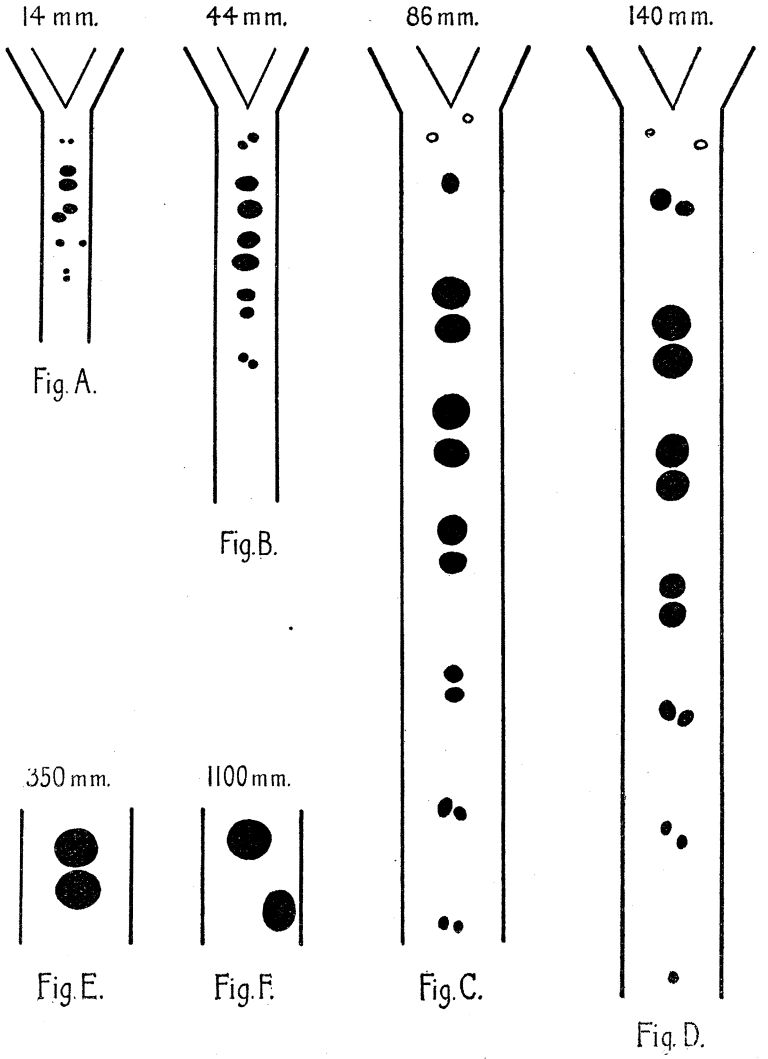
* In the specimens examined about 7 per cent. of the segments which contain giant cells have only one giant cell, instead of the usual couple (see figs. C, D, G, I), and, conversely, I have once seen four large cells, placed close together, one behind the other.

† The two cells of a couple are seldom separated, in an antero-posterior direction, by an interval greater than about one-fifth the length of a segment, and sometimes they are so close together that their sheaths are not only in contact but the cells mutually compress each other, and the faces in apposition are consequently flattened.

‡ In a few cases the posterior of the two cells is partially within the segment behind the one in which the anterior cell is situated. See, for example, fig. 52.

§ These measurements are from preserved specimens, both of which are in a state of moderate extension.

which measures 86 mm.* in length, shows that, compared with the two specimens just described, the number of giant cells has been augmented by the extension



FIGS. A, B, C, D.—Plans of the Nerve Cord in the first Seven Segments of Specimens of *Halla parthenopeia*, showing the Relative Position and Size of the Giant Cells. The primary giant cells are drawn in solid black, the secondary giant cells are shown in outline. The length of the specimen, measured in the preserved condition, to which each cord belongs is stated above each plan. Constructed from series of transverse sections. × 40.

FIG. E.—The Largest Cells—the Second Couple of Primary Giant Cells. From a specimen 350 mm. long when living.

FIG. F.—The Largest Cells—the Third Couple of Primary Giant Cells. From a specimen 1100 mm. long when living.

backwards of the segmental series and by the addition of two small giant cells, right and left, at the anterior end of the nerve cord, near the point at which the

* Although this specimen is considerably shorter than the one described immediately afterwards, it has as many, or even a few more, segments, namely 237. It is the only specimen examined which was preserved simply in alcohol, and its middle and posterior segments seem to have become fixed while in a

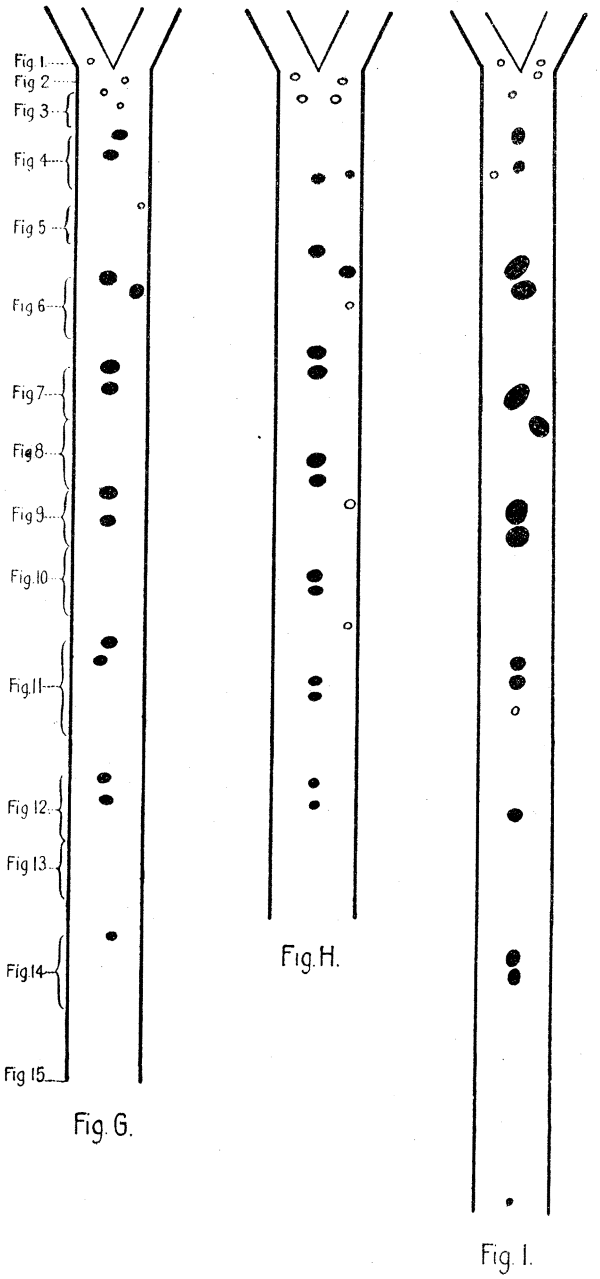
œsophageal connectives enter it. There are, in this specimen, undoubted giant cells in each of the first six chætigerous segments—a single one in the first segment, a couple in each of the five following ones, and there are two cells in the seventh segment which are, almost certainly, young giant cells. Thus there are fifteen giant cells in this worm (see fig. C). The same number of giant cells is present in a specimen 140 mm.* long, which has about 230 segments. There are couples of giant cells in the first six chætigerous segments, a single cell in the seventh segment, and two cells, one right and one left, at the anterior end of the ventral nerve cord (see fig. D). In full-grown worms there are six, seven, or eight segmental couples of giant cells. Out of twenty-two adult examples examined in regard to this point the couples of cells extend backwards to the sixth chætigerous segment in two specimens, to the seventh in eighteen, and to the eighth in two (see fig. I). In none of my specimens do the giant cell couples extend further back than the eighth chætigerous segment.

In front of the segmental couples, at the anterior end of the nerve cord, that is, near the anterior border of the first chætigerous segment, there are two or three giant cells which are almost invariably small (see below, p. 452), even in the largest worms. Although these cells are usually situated in the nerve cord, and not in the connectives, they are nevertheless in intimate association with the nerve elements of the latter, that is, with the fibres which pass from the connectives into the nerve cord. In place of the two or three cells usually found in this region in adult worms, there is in one case only one and in four cases four cells (see figs. G, H, I). Occasionally one (rarely two) of these giant cells is rather farther forwards, and lies actually in one of the connectives (see figs. G, I).

In about one-half of the specimens examined there are one or two giant cells which, instead of being nearly median in position, are found in the right or left lateral group of cells of the nerve cord; in two specimens there are four lateral cells, in another five (fig. H), and in another six. These lateral cells belong to two different categories. A considerable proportion of them, almost one-half of those observed, are found to occur in segments in each of which only one median giant cell is present, and the transverse planes in which the lateral and median cells are situated correspond in their position, both with regard to each other and with regard to the posterior limit of the segment, to those of the cells of an ordinary couple (figs. G, H, I). The lateral and median cells are, in these cases, therefore really the two cells of a couple, one of which has been differentiated in the median group of cells and the other in one of the lateral groups of cells, an inference which is supported by the fact that the lateral cell agrees very closely in size with the median cell with which it forms a

state of considerable contraction, as is evidenced by the unusual breadth of these segments and their short antero-posterior extent. The anterior segments of the worm are moderately extended; were all the segments similarly extended, the specimen would measure about 130 mm. in length.

* This measurement is from a preserved specimen which is in a state of moderate extension.



FIGS. G, H, I.—Plans of the Nerve Cord in the first Eight Segments of full-grown Specimens of *Halla parthenopeia*, showing the Relative Position and Size of the Giant Cells.

FIG. G is from a specimen 30 in. (760 mm.) in length when living. The references to the left of this figure indicate the portions of the cord comprised in each of the reconstructions shown in figs. 1–15 (Plates 32 and 33).

FIGS. H and I are from specimens of which only the anterior ends were captured. The former (H) is remarkable for the number of lateral primary and secondary giant cells, the latter (I) is one of the two specimens in which I have found a giant cell in the eighth segment.

The primary giant cells are drawn in solid black, the secondary giant cells are shown in outline. Constructed from series of transverse sections, × 20.

couple. But other lateral cells observed, altogether about twenty in number, cannot be explained in this way, because they occur in segments which also possess the usual couple of giant cells. In these cases the lateral cell is markedly smaller than those of the neighbouring couples: while the latter range from 100 to 130 μ in diameter, the lateral cells of the same segments are, with one exception, only 30 to 50 μ in diameter. Moreover, these small cells are generally found in the anterior or middle region of the segment, while the large lateral cells above described are invariably situated in the posterior part of the segment to which they belong (see figs. G, H, I). The small lateral cells, therefore, do not fall into series, either as regards position or size, with the ordinary median giant cells and, as they are not present in the four young specimens 14 to 140 mm. in length, and are so much smaller than the cells of the segmental couples, it may be concluded that they arise at a comparatively late stage of the growth of the worm. Those near the connectives arise only slightly earlier, and both these and the small lateral cells may be termed secondary giant cells in contradistinction to the segmental couples, which are the primary giant cells.

Both types of lateral cell are well seen in one of the specimens with twenty-one giant cells (fig. H), five of which are lateral; two of these are posteriorly placed in their respective segments, and are primary giant cells, since they are large and are members of segmental couples; but the other three, which occur in the anterior portion of segments which have in addition the usual couple, are secondary giant cells. It is noteworthy that these secondary cells are almost constantly laterally situated, for in all the specimens examined only four medianly placed secondary cells have been met with (fig. I).

From the foregoing observations the order of the appearance of the giant cells seems to be somewhat as follows: The first giant cells arise in segmental couples, one couple in each of the anterior ganglia of the nerve cord. In specimens from 14 to 44 mm. long, five such couples are already present, and as the worm increases in length other couples are formed in the succeeding segments until a maximum of eight couples is attained. These, which may be called the primary giant cells, have a definite situation in the posterior portion of the segments to which they belong. While the last three couples of giant cells are being formed, there are from two to four smaller secondary giant cells differentiated at the anterior end of the nerve cord, close to the points of entry of the œsophageal connectives, and in a considerable number of specimens one or more small secondary cells appear in the anterior or middle portion of other ganglia already possessing primary giant cells. By these augmentations the number of giant cells in well-grown specimens usually reaches from fifteen to eighteen, but I have one example with twenty (fig. I) and two with twenty-one cells each (fig. H).

II. The Size of the Giant Cells.

The size of the giant cells varies greatly even in the same individual. The secondary cells, situated in the first ganglion near the entry of the connectives, are small, their mean diameter* ranging from about 35 to 55 μ; in only one specimen† is a cell of moderate size (90 μ in diameter) present in this region. The cells of the first segmental couple are considerably larger, averaging about 80 μ in diameter, but those of the second and third couples are usually the largest, though, occasionally, those of the fourth couple are almost equally well developed (see figs. C, D, G, H, I). The largest cell I have seen has a mean diameter of 150 μ; the average diameter of the largest cell of thirty full-grown worms is 132 μ. The cells of the couples behind the third or fourth exhibit a progressive diminution in size. Those of last or most posterior couple sometimes appear as if they had been only recently differentiated (see above, p. 447), in which case they are only about 30 to 40 μ in diameter, but usually they attain a diameter of 50 to 85 μ. The two cells of a couple are generally approximately equal in size.

The secondary lateral and median giant cells, as already remarked (p. 451), are usually small, being about 30 to 50 μ in diameter.

As the worm grows in length there is a progressive increase in the size of the primary giant cells up to a certain point, after which little or no further enlargement of the giant cells seems to take place, however long the worm may become. In the young specimen 14 mm. long (with ninety segments) the mean diameter of the four largest cells is 48 μ (fig. A); in an older specimen 44 mm. long (with 126 segments) the corresponding measurement is 71 μ‡ (fig. B). The four largest cells of the specimen 86 mm. (fig. C), and those of the one 140 mm. (fig. D) in length (both of which have approximately the same number of segments,§ namely 236 and 230 respectively) are nearly equal in size, their mean diameters being 110 μ.‡ The corresponding measurement of those of a worm which, when living, was 350 mm. in length (with about 400 segments) is 135 μ‡ (fig. E).

When the worm has attained a length of 30 to 40 cm., the giant cells seem to have reached their maximum size, for the cells of specimens 50, 60, and 110 cm. in length are no larger than those of the worm 35 cm. long (*cf.* figs. E and F). The increase in size of the giant cells in *Arenicola* is also limited in a similar manner,

* The internal diameter of the cell is given in this and the subsequent measurements of the giant cells, that is, the sheath of the cell is not included.

† Out of thirty full-grown examples the giant cells of which have been measured in order to obtain the data given in this section.

‡ It may be noted that the increase in the volume of the giant cells is roughly proportional to the increase in the length of the worm (except in the case of the specimen 86 mm. long, regarding the length of which see footnote on p. 448).

§ For a note explanatory of the disparity in length of these two specimens, with almost the same number of segments, see p. 448, footnote.

the giant cells appear to attain their maximum development long before the worm has reached its maximum length. For instance, the giant cells in *Arenicola marina* are already well marked in specimens 17·5 mm. in length, and they are apparently no larger in specimens 250 mm. long than in others only one-fourth this length (ASHWORTH, 1904, p. 51).

III. The Number, Arrangement, and Size of the Giant Cells of Chætopoda.

Number and Arrangement.

There are considerable differences in regard to the arrangement of the giant cells present in various Polychætes. In some cases these cells are distributed along the whole length of the nerve cord and do not exhibit any special arrangement. Such is the condition in *Clymene* and *Axiothea*, in which LEWIS could find no evidence of the segmental arrangement of the giant cells which are situated, without any ascertainable regularity or symmetry, along both sides of the nerve cord, and vary in number in different segments; for instance, twelve and eight were counted in successive somites. In other cases the giant cells, while present along the whole length of the nerve cord, exhibit a definite metamerism as, for instance, in *Arenicola*. In the ecaudate species of this genus, in which the external segmentation of the animal is well marked throughout, and in which the parapodia extend to the posterior end of the body, the giant cells are found at regular segmental intervals, there being usually two, one behind the other (or sometimes only one cell) in each segment close to its posterior border (see GAMBLE and ASHWORTH, 1900, Plate 29). In those species of *Arenicola* in which the segmentation of the posterior region or "tail" is feebly marked the giant cells in this region are sparsely and irregularly distributed, but in the anterior parapodia-bearing region they are found at regular segmental intervals (ASHWORTH, 1904, fig. 52).

In other Polychætes the giant cells are present only at the anterior and posterior ends of the nerve cord as in *Halla*, *Aglaurides*, and *Sthenelais*. SPENGEL observed that there are two or three giant cells in each of the first seven or eight ganglia of *Halla*,* and, as shown above (pp. 447–451), the primary giant cells of this worm are found in the posterior portion of each of the first five to eight segments, that is, they occupy in each segment a position homologous with that of the giant cells of *Arenicola*. The anterior giant cells of *Aglaurides* (see below, p. 491) have a corresponding position and arrangement, and the posterior giant cells of *Halla* and *Aglaurides* are also segmentally arranged.

The giant cells described by ROHDE (1887, pp. 43–45) in *Sthenelais* are also divisible into two series. The anterior series consists of a pair in the brain, a pair in the commissural ganglion, and five pairs in the sub-œsophageal ganglion, the

* HASWELL does not indicate that the eight or ten giant cells observed by him in *H. australis* have a segmental arrangement.

processes of all of which run posteriorly, some of them reaching almost to the end of the body. The posterior series comprises a pair of giant cells in each segment except the first sixteen, and the processes of these cells run anteriorly. In *Sthenelais*, therefore, the posterior giant cells are segmentally arranged, but the anterior ones are grouped chiefly in the sub-œsophageal ganglion. EISIG (1887) found four to six giant cells in each ganglion of *Mastobranchus*, but states that in *Notomastus* and *Dasybranchus* they occur chiefly in the anterior region of the animal. SPENGEL recorded the presence of giant cells in the anterior ganglia of *Arabella* (p. 39), and found two giant cells in the anterior ganglion of *Nephthys* (p. 40), and the latter cells were also observed by PRUVOT (1885, p. 239).

The information available regarding the number and arrangement of the giant cells in earthworms is scanty, and all that is certainly known (FRIEDLÄNDER and CERFONTAINE) is that the middle giant fibre arises from an anterior giant cell and receives processes of other similar cells along its backward course, while the lateral giant fibres arise from cells situated near the posterior end of the cord, and as they run forwards receive processes from other cells of similar nature to those from which they arose.

Size.

The giant cells of *Halla* seem to be the largest recorded in Annelids. Those observed by SPENGEL in *H. parthenopeia* were about 100 μ in diameter, the largest recorded in the present communication and those of *H. australis* (HASWELL) have a diameter of 150 μ. The giant cells of *Aglaurides* do not exceed 93 μ, those of *Arenicola* 80 μ (and most of them are about 50 μ), those of *Notomastus* have a mean diameter of 50 to 60 μ, those of *Clymene* and *Axiothea* 35 μ, and those of *Lumbricus* about 70 μ (according to FRIEDLÄNDER'S drawings).

IV. THE COLOUR OF THE GIANT CELLS OF *Halla*; THE REACTIONS OF THEIR PIGMENT GRANULES COMPARED WITH THOSE OF CHLOROGOGEN GRANULES.

When the nerve cord of fresh specimens* of *Halla* is dissected out and examined from the ventral surface, especially while under slight pressure, many of the giant cells are seen to contain a yellowish substance, which, in most cases, is aggregated into a mass, single, as seen under low power, excentrically placed and generally somewhat cup-shaped, in the concavity of which the nucleus of the cell is situated (see Plate 34, fig. 17). In other cases the yellow substance may be present in two or three masses or in the form of an indefinitely limited zone surrounding the nucleus, or it may be more generally distributed throughout the central portion of the protoplasm, but, almost invariably, it is not present in the peripheral portion of the cell. There is a certain amount of individual variation in regard to the depth of colour exhibited by this substance: in the cells of some specimens it is pale yellow,

* Specimens preserved in formalin may also be used, but, as the nerve cord is not quite as transparent as in fresh specimens, the giant cells are not seen so clearly.

while in those of others it is darker yellow, tending to brown. A similar coloration, but to a less degree, is presented by some of the larger ganglion cells, in which a small mass of yellow substance is seen, generally at the end of the cell opposite the axone. In spite of much time devoted to the investigation of this substance, I have not been able to determine its composition, but the enquiry has yielded several interesting results which may be recorded here.

This pigmented substance is best studied in transverse sections of the ventral region of the worm, from fresh specimens or from material preserved in formalin, cut by means of a freezing microtome. Such sections show that not only is a yellow substance present, in the form of minute granules, in the giant cells and some of the ganglion cells, but that rather larger yellow granules, either isolated or in sparsely scattered clusters, are also present in the cellular parts of the cord. Moreover, dorsal and ventral to the nerve cord, in both cases outside its connective tissue sheath, there are generally seen, in sections, masses of tissue containing a yellow substance which seems to be identical with that in the giant cells and in the nerve cord. These masses are apparently portions of the cœlomic epithelium which have become modified to form chlorogogenous tissue similar to that met with in many other Chætopoda, and, as they contain larger quantities of the yellow material than the giant nerve cells, they are more convenient for the application of reagents and were consequently examined first.

The Chlorogogenous Tissue.

The chlorogogenous tissue around the nerve cord of *Halla* varies considerably in amount in different specimens. It does not form a continuous sheath to the cord, but aggregates of chlorogogen cells are situated at moderately regular intervals along the surfaces of the cord. Most of these cells are elongate and fusiform or clavate, others are shorter and more irregular in outline; they are all arranged with regard to each other so as to form fairly compact masses which are generally associated with blood-vessels.

When examined in the fresh condition the chlorogogen cells of *Halla* are seen to contain a number of globules and granules of various sizes, ranging from minute particles to others about 5 or 6 μ, or occasionally attaining 10 μ, in diameter. Some of these are approximately spherical, but many are less regular in outline and, if closely adjacent, are polyhedral owing to mutual compression. On examining them under high power, some are found to be colourless while others are yellow or brownish. The colourless bodies are moderately refringent, but the yellow ones are only slightly so. The former, when observed under the microscope while they are subjected to pressure applied to the cover glass of the preparation, are seen to be fluid at the ordinary temperature and, on being brought together by this treatment, several may unite to form a single larger globule. On placing sections in absolute alcohol, ether, xylol, or chloroform, the colourless globules gradually disappear, but

their complete disappearance is only brought about by treatment for several hours with these solvents in the cold; on heating the solvents to about 50° C. their action is much more rapid. A similar remark applies to the action on the colourless globules of caustic soda solution (2 to 5 per cent.). When a fresh section is treated with osmic acid (1 per cent. for an hour) a considerable number of the bodies present in the chlorogogen cells become black or dark brown. The yellow granules are still recognisable as such, for their colour has undergone only a slight deepening; it is therefore evident that it is the globules which were previously colourless which have become darkly stained. Their fluidity at ordinary temperatures and their blackening on treatment with osmic acid indicate that the colourless bodies contain olein or oleic acid (or both), as these are the only two substances which possess the physical properties described above and which reduce osmium tetroxide in this way (see MANN, 1902, p. 306, and article "Fett" in Encyklopädie der Mikroskopischen Technik, 1903). LORRAIN SMITH (1907A) has recently shown that, on treatment with an aqueous solution of Nile Blue, fatty acids are stained blue or bluish purple while neutral fat is stained red. Sections of *Halla*, from material recently preserved in formalin, were cut with a freezing microtome; some of them were treated with a solution of Nile Blue, washed in water and mounted in FARRANT'S medium. The fat globules exhibited a wide range of colour; many of them were stained red, others reddish violet, others purple and some were blue. The red droplets consist of neutral fat, for they have exactly the same colour as that assumed by droplets of olein which had been treated with the same stain. The violet ones consist of neutral fat with a very slight admixture of fatty acid, while the purple ones are composed largely, and the blue ones probably entirely, of fatty acid. On the following morning about a dozen other sections from the same batch, which had been lying overnight in weak formalin in a covered vessel, were stained in exactly the same way, but in none of them could droplets stained red be found; they were all blue or bluish purple. This clearly pointed to the fact that hydrolysis of the neutral fat had been taking place during the night and that, by the morning, the droplets consisted entirely of acid fat. Formalin is frequently acid, due to the presence of formic acid (produced by oxidation of a portion of the formaldehyde), and on testing the sample in which the sections had stood overnight it proved to be distinctly acid. The presence of even this small amount of formic acid, acting overnight, had evidently caused hydrolysis of the neutral fat, in the minute and easily accessible globules in the chlorogogen cells, converting it into the corresponding fatty acid. LORRAIN SMITH (1907, p. 417) has pointed out that neutral fat in the tissues is in a very unstable condition and that mere exposure of sections to the carbon dioxide of the air for twenty-four hours is sufficient to change the chemical character of the contained globules of fat, transforming them to fatty acid. In addition to the mineral acids which, as is well known, can bring about this change, he remarked that even acetic acid would do so. The above observations show that very weak formic acid is also

capable of inducing this change. On testing the formalin in which the unsectioned worms had been lying for a week or more, I found that this also was very slightly acid, but not so markedly acid as the previous sample tested. It is therefore certain that hydrolysis of the fat had been slowly taking place for some days and consequently that the chlorogogen cells of the living animal contained a larger proportion of neutral fat than was found in the sections which were examined when freshly cut; indeed it is probable that no acid fat was present in the living chlorogogen cells. The fluidity of the droplets at the ordinary temperature and their reaction to osmic acid show that the neutral fat present consists principally of olein, though it is of course possible, even probable, that the higher fats, stearin and palmitin, are present in a state of solution in the olein, but the chlorogogenous tissue and its contained fat are not available in sufficient quantity to enable me to definitely settle this point.*

The yellow granules in the chlorogogen cells are of firm consistency. The largest ones are almost invariably compound, being formed of two or more granules closely apposed. In a few of the sections, examined in the fresh condition, some of the larger granules were seen to be surrounded by a colourless and moderately refringent envelope, the optical properties of which closely resemble those of the fat globules present in the same cells. On treatment with warm absolute alcohol the refringent covering disappeared and the outline of the yellow granule could be more sharply focussed. It may be concluded, from this and other observations, that the clear envelope consists of fat† similar to that of which the colourless refringent globules are composed.

The yellow granules (in fresh sections), on treatment with osmic acid, were only slightly affected: they became rather darker, assuming a light brown colour. Several fresh sections were then stained in a saturated solution, in 70-per-cent. alcohol, of Scharlach R or Sudan III for about twenty minutes, washed in 70-per-cent. alcohol for about the same length of time, transferred to water and mounted in

* The only investigation known to me in which a comparatively large amount of fat from an Annelid has been analysed is that of WILLEM and MINNE (1900), who enquired into the constitution of the fat of *Lumbricus*. They found (p. 38) the proportions of the different fats present to be—butyrin, 4·47 per cent; olein, 87·47 per cent.; stearin and palmitin, 8·11 per cent. They remark that the olein, which so greatly preponderates in this and in the fat of other cold-blooded animals which they investigated, serves to dissolve the less fluid fats, and the mixture remains fluid at the temperature of the organism. The olein present in *Lumbricus* is chiefly located in the intestinal epithelium (p. 45); there are no separate fat globules in the chlorogogen cells of this worm (p. 9).

† A similar observation in regard to the excretory granules in certain leeches has been made by GRAF (1898 and 1899), who states that the free excretory cells (excretophores), for instance in *Nephelis*, contain large numbers of yellow drops, composed partly of oil or fat, in the middle of each of which is a number of small dark granules. He considers that this fluid, which the cell-protoplasm secretes around the granules, serves to isolate these insoluble excretory granules from the protoplasm (1898, pp. 83–85; 1899, pp. 324, 325).

glycerine or in FARRANT'S medium. As similar results were obtained with both stains, only those relating to the former, which are somewhat sharper, need be described. On examining these sections it is found that the colourless, and usually also the yellow, bodies present in the chlorogogen cells have become stained: the former are now bright scarlet, the latter light red. Though the latter have lost, during their twenty minutes' washing in alcohol, a portion of the red colour which they possessed on being taken out of the stain, they tenaciously hold against all washing sufficient red colour to show that, associated with the yellow granules, there is a substance on which Scharlach has a selective action: nothing in the tissue of the sections retains the red stain except the fat globules and the yellow granules present in the chlorogogen cells, in the nerve cord, and in the giant and ganglion cells. In view of the well-known selective action of this dye for fats it seems probable that the substance in question is of a fatty nature. The complete removal of this stainable substance requires prolonged action of a solvent in the cold, at any rate in some cases, for even after sections have stood in absolute alcohol for several hours the yellow granules will still stain with Scharlach, but not so red as before. But on warming sections in any fat solvent (absolute alcohol, ether, chloroform, etc.) the substance which stains is soon dissolved, and subsequent treatment with Scharlach produces no reaction, the granules remain a yellow colour and exhibit no trace of red staining. A similar result is attained by treating the granules, in a fresh section, with warm caustic soda solution; they will no longer stain with Scharlach. From the above observations it is justifiable to conclude that fat is usually associated with the yellow granules; either they contain fat or, possibly, are only enveloped in a thin film of it. Although, owing either to the minute proportion in which the fat is present, or to its chemical nature, it does not give a definite reaction with osmic acid, its presence is demonstrated by the two facts—(1) the staining of the yellow granules with Scharlach or Sudan, and (2) that it is only necessary to previously "extract" the granules with any of the usual fat solvents or to treat them with warm solutions of the caustic alkalies in order to render negative their reaction to these stains. The proportion of fat associated with the yellow granules varies widely in different specimens, but less so in regard to the granules in the various cells of the same specimen. Occasionally granules are seen which do not react with Scharlach, others show a weak reaction, but generally the reaction is quite clear.

The yellow chlorogogen granules remain undissolved even after lying for one or two days in absolute alcohol, ether, chloroform, or xylol, all maintained at 50° C., but their colour is paler than at first. Caustic soda or potash solution, 5 per cent., acting for three days, has no solvent action upon the substance of the granules, but removes some of their colour. Even at the end of several minutes' heating in these solutions of the alkalies many yellow granules were still present, although the whole of the tissue was becoming very soft and beginning to break up. The granules are insoluble in glacial acetic acid and in concentrated sulphuric hydrochloric or nitric

acids, but these mineral acids darken the granules, especially the larger ones, so that they become light brown or brown in colour, but they do not assume a blue or green colour as do many lipochromes on treatment with concentrated sulphuric or nitric acids. These reactions indicate that the substance of which the yellow granules are composed is of a resistant character, and it is apparently similar to that found in the excretory cells of Annelids by other workers. WILLEM (1899, pp. 556–559), who made a careful study of the chlorogogen cells of *Arenicola marina*, found them to contain droplets of fat (olein) granules of acid urate of sodium,* and other granules which were insoluble in ammonia and in strong solutions (even hot) of caustic potash. As he remarked, these resistant granules approach in their insolubility certain concretions found by EISIG (1887) in the blood-corpuscles (p. 718), nephridia (pp. 729, 730) and chlorogogen cells (p. 756) of Capitellids, and by SCHAEPPI (1894, p. 284) in the chlorogogen cells of *Ophelia radiata*. Similar insoluble yellow granules were found in the middle (excretory) portion of the nephridium of *Arenicola* by WILLEM (pp. 750, 751), and by GAMBLE and ASHWORTH (1900, p. 516), and the latter observers also found them in some of the cells of the heart body of this worm (p. 464). EISIG and SCHAEPPI considered these insoluble granules to be formed of chitin, but WILLEM (p. 559, footnote), who shows that their reactions do not coincide with those of chitin, was unable to determine their nature. Although the insoluble yellow granules in the chlorogogen cells of *Halla* are resistant, and in this respect remind one of chitin, their chemical reactions (for instance, on treatment with iodine and zinc chloride) do not agree with those of this substance, nor does their behaviour (described below) on long immersion in alcohol or ether agree with that of chitin, but, like WILLEM, I am unable to determine of what material they are composed.

On prolonged treatment with alcohol these granules become disintegrated, and to a large extent dissolved. On examining the chlorogogenous tissue of specimens which had been kept in alcohol (80–90 per cent.) for two years, the chlorogogen cells are found to contain no yellow granules, such as are seen in the fresh tissue, but instead they contain some minute colourless granules which are either the remains of the large granules or, more probably, the ordinary cytoplasmic granules; the chlorogogen granules have largely or totally disappeared. A similar effect can be brought about in a shorter time by keeping sections of the chlorogogenous tissue in absolute alcohol or in a mixture of absolute alcohol and ether maintained at 55° C. After three days of such treatment with warm absolute alcohol the granules are paler in colour than when fresh; many are still yellow, but others are colourless or almost so. On the fourth day the granules exhibit a tendency to break up; some of them are, in fact, falling to pieces. Those granules which retain the yellow colour seem to be more resistant to disintegration than the colourless ones. After eleven days in warm

* The chlorogogenous tissue of *Halla* is not available in sufficient quantity to enable me to test, with much hope of a definite result, for the presence of urate of sodium, and I have not attempted to do so.

absolute alcohol there are no large granules left in the chlorogogenous tissue of one specimen; they have broken down into smaller particles, and a diffuse pale yellow colour is seen in places. In another specimen the changes have not proceeded quite as far: chlorogogen granules, some of which are yellow, are still present, but they are obviously tending to early disintegration. Similar effects may be more rapidly produced by a mixture of absolute alcohol and ether, for after lying in this mixture, kept at 55° C., for six to eight days the chlorogogen granules, after first becoming dissociated into small particles, have in many cases disappeared—that is, have been dissolved, so that the chlorogogen cells present the same appearance as those of specimens kept in alcohol for two years.

The yellow colour of the chlorogogen granules of *Halla*, and the presence in them of fat, at first suggested that their pigment is a lipochrome, especially as MacMunn (1889, p. 75) found a colouring matter of this nature in the chlorogogenous tissue on the "stomach" of *Arenicola*. But the pigment of these granules in *Halla* does not turn blue or green on treatment with concentrated nitric or sulphuric acids, and is only very slowly dissolved by the usual lipochrome solvents; moreover, the fat may be totally removed so that the granules no longer react to Scharlach and yet a considerable amount of the yellow colour still remains in the granules. These facts seem to indicate that the pigment is not an ordinary lipochrome.* The colouring matter has a great stability and a degree of resistance to solution almost as high as that of the substance which it impregnates. Eisig (1887, p. 729) remarked upon the similarly resistant character of the colouring matter found in the nephridial and chlorogogen concretions of *Clistomastus*, and considered that it was probably derived from hæmoglobin, although the tests for blood and bile pigments which he applied to these and to similar concretions in the blood corpuscles were negative; the pigment did not show any absorption bands. According to Willem (1899, p. 563) the pigment in the chlorogogen granules of *Arenicola* owes its origin rather to the substances on which the animal feeds than to hæmoglobin. In their resistance to reagents these pigments resemble some of the iron-free pigments found in man.† I have been unable to ascertain the presence of iron in the chlorogogen granules of *Halla*, but, owing to the difficulties of obtaining definite evidence of the presence of organic or "masked" iron in such small quantities, I am not prepared to say that it is absent. Further work on the pigment of the chlorogogen granules of some Annelid, in which there is a larger amount available than in *Halla*, is necessary before its

* Our knowledge of the lipochromes and their derivatives which occur in Annelids is scanty, but it is known that some of the derivatives may possess a degree of resistance to solution considerably higher than that of ordinary lipochromes. For instance, the yellow lipochrome present in the skin of *Arenicola* apparently readily undergoes modification into a substance which is not soluble in the ordinary lipochrome solvents, and which forms the insoluble "melanin" present in the epidermal cells. This dark pigment, according to MacMunn and Fauvel (1899), is probably produced by the action of acids on the yellow lipochrome which is so abundantly present in the same cells.

† See, for instance, H. G. Wells, 'Chemical Pathology,' London, 1907, pp. 403–404.

nature can be ascertained. For the present the character of the pigment of these granules remains undetermined.

To summarise, we may say that the chlorogogen cells around the nerve cord of *Halla* contain: (1) refringent fat droplets in which olein largely predominates; (2) granules which are composed of a resistant substance impregnated with a yellow pigment (which is not an ordinary lipochrome), both of which are of unknown nature, and that, generally, fat is associated, possibly only as an enveloping film, with the granules, some of the larger of which are composite, being formed of two or three smaller granules closely apposed.

The Clusters of Granules in the Nerve Cord.

When sections of the nerve cord, from fresh specimens or from material preserved in formalin, are examined, both colourless and yellow bodies, the latter largely predominating in number, are found in the cellular parts of the cord. These are markedly smaller and more uniform in size than those of the chlorogogen cells, being usually about 1 μ in diameter. The colourless bodies stain darkly with osmic acid, and, as they behave exactly like those of the chlorogogen cells above described, they probably consist largely of olein. In some specimens these fat globules are scarce or even altogether absent. The yellow granules present in the nerve cord are resistant and also correspond in their other reactions to those of the chlorogogen cells;* they are only slightly darkened with osmic acid, but stain quite definitely (as described on p. 458) with Scharlach or Sudan.† Many of the bodies of both kinds are aggregated into clusters in a way which suggests that each group is contained within a cell, and stained preparations show that this is generally the case. On examining sections treated first with Scharlach R (which stains the globules of both kinds red) and then with hæmatein (which stains the cytoplasm and nuclei of the cells) it is seen that some of the cells in the cord are so full of red-stained bodies that, in each case, only a small portion of the cytoplasm remains along with the nucleus (see Plate 36, fig. 44). These cells have not the appearance of ganglion cells, they are apparently specialised small cells whose function is to secrete the granules, that is, they seem to be isolated special cells of practically the same type as the chlorogogen cells. Other granules present in the nerve cord are not intracellular but intercellular, they occur either singly or a few (up to about half-a-dozen) together in the intervals between the ganglion cells. As they are of the same size and nature as those just described, it would appear that they were also originally formed in cells, but either in the course of preparation, or naturally, the granules have become freed from their cells.

* In sections treated with absolute alcohol heated to 55° C. the granules in the nerve cord remain recognisable for three days, although they have by this time lost their colour, but on the fourth day they begin to break down into clusters of very small particles, which subsequently disappear, being probably dissolved.

† Except in one specimen, where they react very feebly.

The Yellow Granules in the Giant Cells.

The yellow granules in the protoplasm of the giant cells are smaller than those previously considered; they are usually minute particles, but a few of the larger ones attain a diameter of 1 μ or rarely 2 μ (Plate 36, fig. 41). They are generally aggregated into one or more masses in the cell, as already described (p. 454, and Plate 34, fig. 17), but a few others may be seen scattered through the protoplasm. These yellow granules are present in both large and small cells, though they are perhaps less abundant in the latter. They darken only slightly with osmic acid, but in most cases stain red with Scharlach. There is, however, considerable variation in this respect; in one or two specimens the granules stain feebly with Scharlach, but in most cases the reaction is quite definite. On treating the giant cells with warm absolute alcohol or any other fat solvent and subsequently staining with Scharlach, no red coloration of the granules can be obtained. The granules and their pigment behave, with regard to various reagents and solvents, very like those of the chlorogogen cells and of the granule-containing cells of the nerve cord, that is, they are insoluble in alcohol, ether, acids, and alkalies when the action of these substances extends over a few hours only, but on more prolonged action of alcohol or a mixture of alcohol and ether the pigment disappears, the granules seem to persist for some time longer, but soon become indistinguishable from the ordinary cytoplasmic granules. Specimens which have been kept for several months in 80 to 90 per cent. alcohol show no pigment in their giant cells. These granules, therefore, are composed of a substance impregnated with a yellow pigment, both of which appear to be closely similar to, if not identical with, those found in the chlorogogen granules, and fat is usually associated with the granules in the giant cells.

The Yellow Granules in the Ganglion Cells.

Minute yellow granules, rarely exceeding 1 μ in diameter, presenting exactly the same reactions to stains and solvents as those of the giant cells, occur in many of the ganglion cells, especially in the larger ones, where they are usually found at the end opposite the axone. In some cases the granules are also present in other parts of the cell (Plate 36, figs, 42, 43).

Bethe (1903, p. 150) states that the pigment granules in nerve cells stain feebly or not at all with basic dyes, and that the stain is removed by passing the tissue through alcohol. Sections of *Halla*, stained with toluidine blue, dehydrated and mounted in balsam, usually show only a feeble staining of the yellow granules in the various cells. Most of these granules are more or less purple in colour, but some are pale blue. The granules of the giant and ganglion cells and those in clusters in the nerve cord react identically to this stain, while those in the chlorogogen cell stain, on the whole, more lightly.

The Pigment in the Nerve Cells of Annelida and Mollusca.

The presence of yellow pigment in the nerve cells of Annelids has long been known, for LEYDIG (1864, p. 153) records the occurrence of a spot or patch (Fleck) of yellow granular substance in the ganglion cells of *Lumbricus*. NANSEN (1887A, p. 111) found fat-containing granules in the nerve cells of *Nereis*, but does not state that they are pigmented. So far as I am aware, no detailed observations on the pigment in the nerve cells of Annelids have hitherto been published, but considerable attention has been devoted to that in the nerve cells of certain Mollusca. LEYDIG pointed out that pigment was present in the ganglion cells either in the form of granules (as in Vertebrates) or of a diffuse yellow substance (for example, in *Paludina*). BUCHHOLZ (1863), who made a careful chemical examination of the pigment bodies present in the nerve cells of *Planorbis* and *Limnæus*, concluded that they are composed of a red unsaponifiable fatty substance. In many of the ganglion cells of *Planorbis* (but not in *Limnæus*) these bodies form an irregular mass at the point of exit of the cell-process. He found that the small ganglion cells are non-pigmented and that the pigment appears later and increases in amount as the cells grow. SCHULTZE (1879, pp. 75, 76) recorded the presence of yellow granules, blackened by osmic acid, in the nerve cells of some Gastropods. HALLER (1886, pp. 339–340) found droplets of various colours, easily extracted by alcohol and browned by osmic acid, in the nerve cells of some Rhipidoglossa (*Fissurella* and *Turbo*), and regarded them as products of metabolism. NANSEN (1887A) carefully described the granules of which the pigment is composed: he noticed that, in the nerve cells of *Patella*, these granules were not only scattered through the protoplasm but were often concentrated in special parts of the cell, especially in the neighbourhood of the nucleus (p. 116). He also found smaller granules outside the ganglion cells in maceration preparations, and concluded that they had either exuded from cells or that the cells in which they were contained had been destroyed. He believed the yellow substance contained fat, that its colour was due to a pigment similar to hæmoglobin, and that it was principally concerned in the nutrition of the cell (an opinion which he erroneously attributed to HALLER). LEGENDRE (1905, 1906) has recently examined the pigment granules in the nerve cells of *Helix*. They are spherical, of variable size, isolated, grouped or irregularly distributed, but found especially in the cone of origin of the axone and also along the course of the axone as far as the neuropile. The granules are, in the fresh condition, refringent and yellowish green, and osmic acid may or may not darken them. The pigment is stated to be a lipochrome. The quantity of granules present is variable, some cells may possess them in abundance while others have none.*

It is evident from a consideration of these memoirs that the colour of the nerve cells of various Mollusca is not always due to the same cause; in some cases diffused

* A mass of dark yellow pigment occurs in the majority of the ganglion cells of *Anodon* (BOCHENEK, 1905, p. 210), but its nature does not seem to have been investigated.

pigment is present, while in others the colour is contained in droplets or granules. Several of the pigments, for example those described by BUCHHOLZ and HALLER, are lipochromes, and, in nearly all cases, fat is in some way associated with the pigment.

Yellow pigment granules have also long been known to occur in the nerve cells of Vertebrates, and have been regarded by many writers as products of metabolism, though other authors do not assent to this view. There is also divergence of opinion as to whether the granules are of fatty nature (see, for instance, ROSIN, 1896, and ROBERTSON, 1899, pp. 232, 233).

The evidence afforded by *Halla*, while not conclusive, because the nature of the substance of which the yellow granules are composed remains undetermined, is, at any rate, suggestive. The substance and pigment of the yellow granules in the nerve cells of *Halla* have been shown to be closely similar to, if not identical with, those of the chlorogogen granules, which are admittedly excretory products, a fact which indicates that the granules in the nerve cells are probably insoluble products of metabolism.

It may be here remarked that, judged by their staining reactions, the granules in the chlorogogen cells of *Halla* do not appear to have a uniform composition, and there is also some variation in regard to the reactions of the yellow granules in the giant cells of various specimens. The association of fat with the yellow granules in the nerve cells of *Halla* appears to be usual, but the amount of fat so present is very variable in different specimens. In most specimens the granules react to fat stains quite clearly, but in at least one specimen the reaction was feeble. It is possible that fat is not an invariable or essential constituent of the yellow granules, that, under certain conditions, it may be altogether wanting, and that, when it is present, it may form merely an enveloping film.

Since the foregoing section of this paper was finished, an important statement on the subject of pigment in Molluscan nerve cells has been published by SMALLWOOD and ROGERS (1908). They found certain dark bodies, many of them in vacuoles, in the nerve cells of *Limax*, and ascertained that these bodies disappeared in fatigued animals (p. 68), but that solid bodies reappeared within an hour or two after the stoppage of the stimulation (p. 69). The golden-brown granules in the nerve cells of *Planorbis* appear to be of a different character. They do not disappear on stimulation of the nerve cells, and in animals starved for long periods the changes in appearance of the nerve cells were very slow. After the animals had been kept without food for three months the pigmented substance in their nerve cells "is in the process of being broken down, it becomes very finely divided, and seems to become actually less in amount" (p. 71). The authors regard the bodies in the nerve cells of both *Limax* and *Planorbis* as a storage product which has to do with the nourishment of the cells when proper food is unavailable (p. 72). Although osmic acid blackens the bodies only after a long time and in many cases only

superficially, and the reaction with Sudan does not appear to have been very definite, the authors conclude that the bodies consist of "some sort of a fat." They find that the bodies swell up in ether, and become gradually but very slowly dissolved and diffused throughout the cell. They regard the pigment as one of the lipochrome group, as the bodies, previously golden brown, assume a bright blue colour on treatment with concentrated sulphuric acid. Bodies of similar nature to those in *Planorbis* were also found in *Limnæa*, but the yellow substances present in the nerve cells of *Venus* and *Melantho* are of a different character to those above described, and have not yet been investigated.

The dark bodies in the nerve cells of *Limax* certainly appear to be of the nature of food reserves which can be readily rendered soluble and serviceable to the cells which contain them, but the brown granules of *Planorbis* are much more resistant, and their behaviour on stimulation of the cell and on long starving of the animal does not seem to conclusively point to their being storage products in the sense of nutritive reserves.

The substance of the granules which occur in the nerve cells of *Halla* is evidently much more comparable in its powers of resistance to that found in *Planorbis*, rather than to that of *Limax*, and it resembles the former in its very slow but eventual solution in ether. The granules of *Halla* differ from those of both these Mollusca in the nature of their pigment, which, on treatment with concentrated sulphuric acid, does not turn blue as in these Mollusca, but darkens in colour to brown.

V. The General Morphology of the Giant Cells of *Halla*.

Their Shape.—The giant cells are seldom spherical; they are sometimes pear-shaped, but more usually their axes are of unequal length. Each cell gives off one large process or axone—the giant fibre.

The Cytoplasm.—In thin sections stained with iron hæmatoxylin, the cytoplasm of the giant cell presents the appearance of an almost colourless matrix in which lie large numbers of small, usually moderately stained rounded granules (Plate 34, fig. 18). In some cases, however, there are deeply stained granules present (fig. 53) which form one or more darker patches in the cytoplasm, the appearance and position of which suggest that they probably correspond to the yellow areas observed in living cells (see p. 454). There seems to be considerable variation in the amount of staining with hæmatoxylin exhibited by these granules: in some specimens they stain lightly, while in others they have a greater affinity for stain, as in the cases above mentioned. Whether darkly stained granules be present or not, there are very numerous moderately stained ones distributed throughout the central portion of the cytoplasm and varying in size from the smallest visible with an immersion lens to a maximum of about 1 μ in diameter. They are fewer in number or are altogether absent in the cone of origin of the axone and in a peripheral zone of the cell-protoplasm, the breadth of which varies somewhat in different specimens (fig. 18).

The granules which stain only moderately well with iron hæmatoxylin are more responsive to methylene blue and toluidin blue. In sections which have been treated with either of these stains many of the giant cells present exhibit fine blue granules which are not arranged in lines or concentric rows or in any other ascertainable order, but are almost evenly distributed throughout the general portion of the cytoplasm (Plate 36, figs. 45, 46). They are present in greatly increased mass in the specialised perinuclear zone which is described below (p. 467), but they are usually absent from, or are very sparse in, the peripheral zone already mentioned and in the cone of origin of the axone, and there are none in the giant fibres. This chromophilous substance, which is apparently equivalent to the Nissl substance of the nerve cells of Vertebrates, is rarely aggregated into flakes as it frequently or usually is in the nerve cells of higher Vertebrates, but is, as already stated, scattered in the form of small rounded granules, the largest of which are about 0·5 to 1 μ in diameter, throughout the central portion of the cytoplasm. As one would expect, the amount of this chromophilous substance varies considerably in the cells of different specimens and even in the several cells of the same individual, a variation which is no doubt dependent upon the functional condition of the cell just previous to the time of fixation.

When stained with toluidine blue and erythrosin (Holmgren, 1900, p. 37), the cytoplasmic matrix of the giant cells assumes a pale pink or faint purple colour and in it lie the granules, most of which are stained blue or bluish purple (figs. 47–50). In some cells, however, a few clusters of more acidophile granules are present in the general cytoplasm, especially towards its margin, so that reddish purple or red granules are sometimes met with close to the peripheral zone.

The peripheral zone of protoplasm is invariably faintly stained, in some cells it is pale pink (fig. 47), in others pale blue (fig. 49) or a faint purple. The protoplasm of the giant fibre and of the cone of origin of the axone stains very similarly to that of the peripheral zone, except that that of the cone of origin of the axone is sometimes slightly more deeply stained. The peripheral zone of protoplasm is not differentiated in the giant cells of the specimen 44 mm. long (see Plate 33, fig. 16) and is only feebly marked in those of the specimen 86 mm. long, but it is almost invariably clearly marked in both the small (figs. 46, 49) and large cells of adult specimens. Only two or three cells have been met with in adult specimens in which this zone is not differentiated, one of them is shown in fig. 50.

The sheath of the giant cell, in preparations stained with toluidin blue and erythrosin, is bright red and its nuclei are blue (figs. 47–50). Here and there elements of the sheath extend into the peripheral zone of protoplasm of the giant cell (see below, p. 481).

Vacuoles.—In two or three cells only, out of many scores examined in section, the protoplasm is non-vacuolated (Plate 36, fig. 50). As a rule vacuoles, with sharply marked outline, are present in varying number in different cells in the neighbourhood

of the nucleus, they are almost invariably absent from the peripheral zone and from the cone of origin of the axone (Plate 34, figs. 18, 19; Plate 36, figs. 45–49). In a few of the specimens examined the central portion of the cytoplasm is so extensively vacuolated that it has assumed an almost spongy appearance. This is the case in some of the cells in the young specimen 44 mm. long (Plate 33, fig. 16) as well as in those of some adult examples. This extreme vacuolation is probably due to recent excessive activity of the cell. The vacuoles contain a coagulum which, with erythrosin, stains sometimes feebly but in other cases quite red, in a few cells even a deeper red than the cytoplasmic matrix of the cell, but with iron-hæmatoxylin is usually faintly stained, only occasionally is a more deeply stained mass present. This coagulum is occasionally granular (fig. 47). Several of the giant cells show structures which resemble the intracellular "Saftkanälchen" described by HOLMGREN (1900) in the nerve cells of *Astacus*, *Hirudo*, *Helix*, etc., and regarded by him as trophic in function. These canals are seen, in the giant cells of *Halla*, running from the glia sheath of the cell well into the cytoplasm. Many of them enter the cell along with a cluster of fibrillæ (probably glia fibrils, see p. 482).

The Perinuclear Zone of Cytoplasm (Plate 34, figs. 18, 19; Plate 36, figs. 45–50; Plate 37, figs. 53–56).—On examining fresh preparations (Plate 34, fig. 17) or nerve cords stained and mounted entire, the nucleus of many of the giant cells appears to be surrounded by a refringent envelope which may readily be mistaken for a thick double-contoured nuclear membrane, as it was described by SPENGEL (1881, p. 38), but when thin sections are examined this interpretation is seen to be erroneous. The nuclear membrane is thin, but is surrounded by a zone of dense protoplasm the refringency of which is considerably greater than that of the remaining protoplasm, and is responsible for the appearances above described in the cells examined entire. This zone is frequently very sharply marked, its outer margin is limited by a close network of neurofibrillæ (the perinuclear network, described below, p. 478), which can be seen in fresh preparations in which, in optical section, it forms the outer contour of this refringent envelope around the nucleus, the inner contour being formed by the nuclear membrane. In many cases this network of fibrillæ and the nuclear membrane form, as seen in section, two almost accurately concentric circles, the intervening zone of protoplasm being regular, or nearly so, in width in all its parts. In these cases the zone, which is from 3 to 5 μ in width, stains very darkly with iron-hæmatoxylin, but small vacuoles are seen in it at intervals (fig. 19). In preparations stained with toluidin blue and erythrosin the perinuclear zone assumes a deeper shade of the same colour (blue or bluish purple) as the rest of the protoplasm and its vacuoles are well seen because of their red-stained contents. Occasionally a giant cell is seen in which the perinuclear zone, while quite sharply limited over the greater part of its periphery, sends out, at one or more points, irregular deeply-stained offshoots which merge into the general, more lightly-stained

protoplasm of the cell (Plate 36, fig. 48). In a few other giant cells the zone is considerably enlarged, and then exhibits a somewhat diminished affinity for stains, except close to the nuclear membrane, but even at its periphery it is moderately sharply defined from the general cytoplasm (fig. 53). In several cases the zone, instead of being concentric with the nucleus, is ovoid, and the nucleus is situated in its broader end, but the narrower end is not necessarily directed towards the axone (figs. 53, 54). In a few cases, and apparently more frequently in the case of small giant cells, the zone of protoplasm between the perinuclear net and the nuclear membrane is not so markedly differentiated by its staining reactions from the rest of the cytoplasm, and in two or three cases (fig. 50) no definite perinuclear zone is recognisable.

No other nerve cells known to me present such a sharply-marked perinuclear zone as that seen in *Halla*. The cells of *Halla australis* evidently resemble those of *H. parthenopeia* in this respect, judging from HASWELL'S figure (1886, Plate 55, fig. 4) of one of the former.

The more deeply-staining character and denser appearance of the perinuclear zone above described are due to the presence within it of numerous bodies apparently similar to the blue- or purple-stained (with toluidin blue and erythrosin) granules present in the general protoplasm of the cell, but the former are rather larger and more chromophilous. It is worthy of note that, if there be any differential staining within the zone itself, the most deeply-stained portion is invariably that directly applied to the nuclear membrane. Vacuoles, similar to those in the general cytoplasm, are found in this specialised zone, and their coagulated contents are usually stained red with erythrosin.

In the two or three cells above mentioned (p. 466), in which the protoplasm is non-vacuolated, there is no clearly-marked perinuclear zone. The most deeply-staining portion of the protoplasm is that directly applied to the nuclear membrane, but this shades off gradually into the general cytoplasm, so that there is no definite perinuclear zone (fig. 50). The granules in these few cells are apparently less basophile than in most of the cells examined.

Several investigators have recorded the presence of nuclear derivatives in the cytoplasm of nerve cells. ROHDE (1896) described and figured a ganglion cell of *Helix* from the nucleus of which processes, unlimited by a membrane, extend into the cytoplasm, and also a ganglion cell of *Doris* in which there is no sharp line of demarcation between nucleus and cytoplasm, but the nuclear substance gradually merges into the cytoplasm. In a later paper, ROHDE (1903) maintains that in the nerve cells of various Vertebrates there is a migration into the cytoplasm of basophile substance from the nucleus when it exists in the nuclear sap either in solution or in a diffuse condition. HOLMGREN (1900) states that one of the signs of activity in the nerve cells of *Lophius*, *Gadus*, and *Gallus* is an out-streaming of nucleoli or other nuclear constituents into the cell plasma, where they often

exhibit a somewhat radiate arrangement* of basophile and acidophile granules. It seems to be now established that the Nissl bodies of the nerve cells of Vertebrates consist of nucleo-protein. SCOTT (1899) traced the origin of the chromophile substance, and concluded that the chromatin of a young nerve cell divides into two portions—one, oxyphile, which remains within the nucleus, and the other, basophile, which diffuses, probably not as actual particles but more probably in a state of solution, into the cytoplasm, when it becomes transformed into the Nissl granules. CAMERON'S observations (1906) lead him to conclude that the chromatin material is not discharged from the nucleus in the form of granules, as described by HOLMGREN, but is first transformed into an achromatic substance which is extruded from the nucleus, probably in a fluid state, and very soon undergoes a process of rechromatisation, giving rise to the Nissl bodies and also to the neurofibrillar network. HATAI (1904) concludes that the Nissl granules, when first formed in the spinal ganglion cells of the rat, are derived either by diffusion of nucleins from the nucleus or by a migration of the accessory nucleoli into the cytoplasm.

The perinuclear zone in the giant cells of *Halla*, which is due to the heaping-up of chromophilous material around the nucleus, and especially its concentration in that portion of the zone directly applied to the nuclear membrane, seems to support the chemical and morphological observations cited above, and to clearly indicate that there is a diffusion or extrusion from the nucleus of some substance which is rapidly converted into the basophile granules present in this specialised zone of protoplasm I have not met with anything resembling the radiate arrangement of basophile and acidophile granules around the nucleus, which was regarded by HOLMGREN as an expression of the outward diffusion stream from the nucleus, nor have I found the nuclear membrane to be incomplete, as appears to be the case in several of the cells figured by him at the point where the radiations are most pronounced (see his figs. 29, 55, 56, 60, 61). The appearances presented by the giant cells of *Halla* suggest that the substance diffuses outwards practically evenly from the whole periphery of the nuclear membrane, which is everywhere complete. There is usually also a similar equal distribution of the chromophilous substance from the pepiphery of the special zone into the general cytoplasm, but occasionally, as already mentioned, offshoots of this material extend outwards into the general cytoplasm at one or more points. The perinuclear network, which is closely associated with, and bounds the outer edge of, this specialised zone of cytoplasm, is generally the most obvious part of the neurofibrillar network in the cell by reason of its close meshes and somewhat stouter strands. Whether the association of the well-developed network with this zone, into which nuclear substance is being constantly extruded, is to be ascribed to the origin of its constituent neurofibrillæ from such extruded material, as concluded by CAMERON, I am unable to say, but the

* A similar appearance has been observed by SMALLWOOD (1906, p. 185) in the nerve cells of the Opisthobranch *Haminea*.

relations of the perinuclear net to the nucleus would certainly appear to facilitate rapid and extensive nutrition of the network, and may be taken as indicating that the latter plays an important part in the special functions performed by the cell. Regarding the difficult question as to whether the substance diffuses from the nucleus in the form of chromatin particles or as a fluid, it may be remarked that the equal diffusion from the whole periphery of the nucleus through the imperforate and intact nuclear membrane seems more compatible with the view that a liquid substance is involved. The granules within and immediately without the nucleus are separated only by a membrane of great tenuity, about 0·2 μ in thickness, but they differ markedly in their staining reactions: the chromatin particles within the nucleus stain red (acidophile), while the granules just outside the membrane stain blue or bluish purple (basophile) when treated with toluidin blue and erythrosin. The change from the one to the other may be accomplished by the formation of some intermediate diffusible product, as seems to be suggested by several of the observations noticed above, particularly those of ROHDE (1903) and CAMERON.

The Nucleus.—The nucleus of the giant cell is usually near the centre of the cell, or slightly nearer its broader end. It is, like the nuclei of most nerve cells, large and vesicular. In small giant cells 40 to 60 μ in diameter, the nucleus is 16 to 20 μ in diameter, in cells of moderate size it is 30 μ, and in the large cells, about 130 μ in diameter, the nucleus measures as much as 40 μ across. The nuclear membrane is acidophile, thin (in the cases measured it is about 0·2 μ in thickness), and complete (figs. 47–50). The chromatin, which may be either rather scanty or moderate in amount in different cells, is in the form of minute acidophile granules arranged on the threads of an achromatic (plastin) network (figs. 18, 19).

The Nucleolus (figs. 19, 20, 24, 47, 50, 51).—There is invariably only one nucleolus in each giant cell. It is large, 5 to 7 μ in diameter in medium-sized and large cells, and is usually moderately deeply stained with iron-hæmatoxylin. In many cases the nucleolus is divisible into two portions differentiated by their staining reactions; it contains three or four more or less globular bodies which are acidophile, but not very strongly so, as they often stain quite lightly, placed in a matrix of basophile substance; or, to state this in another, and perhaps more accurate, way, the acidophile bodies have each an envelope of basophile material, and there is a common envelope of the latter substance around the whole.* In sections stained with toluidin blue and erythrosin the envelope of the nucleolus is stained blue, and its interior is crossed by three or four blue divisions, in the spaces between which are the bodies stained red or pink. In other nucleoli the two substances are apparently blended.

It may be noted here that in none of the cells examined have I seen a centrosome

* For a discussion of the nature of these so-called double nucleoli, see the recent memoirs of MCGILL (1906) and HEIDENHAIN (1907, pp. 181–189).

and radiations, indicating a sphere, such as were described by LEWIS (1898) in the giant cells of *Clymene producta*.

VI. The Course of the Giant Fibres of *Halla*. (Plates 32 and 33.)

Each giant cell gives off one stout process—the giant fibre. The giant fibre which issues from a giant cell situated in the mid-ventral group of cells in the nerve cord runs obliquely dorso-laterally, through the fibrous part of the nerve cord, until it almost reaches the lateral cell group; it then crosses to the opposite side of the cord, and gradually curves inwards to its final position in or near the middle line. During the course just described the axone, from its exit from the giant cell to the attainment of its final, almost median, position, will have traversed the greater part of a segment. The giant fibres of the neighbouring cells of a couple cross in or near the middle line, and often lie in intimate association, sometimes for a considerable distance (30 μ), almost suggesting an anastomosis, but no such connection could be ascertained. In the case of a giant cell situated in the lateral group of ganglion cells of the nerve cord (fig. 6) the giant fibre issues from the cell on its median aspect, enters the fibrous part of the cord, traverses it to the opposite side, generally running directly across the cord, and then curves gradually inwards and attains a position alongside the other giant fibres from the giant cells of adjacent ganglia. The giant fibres from the first eight or ten giant cells finally come to lie in the dorsal part of the nerve cord just within its tough envelope, the giant fibres from the remaining (more posterior) giant cells are usually situated more ventrally, nearer the middle or in the mid-ventral portion of the fibrous part of the cord, but in many specimens they also, after running through a variable number of segments, gradually turn dorsally and are found close to or among the fibres from the anterior giant cells.

In many specimens the processes of successive giant cells, especially those of the primary cells, pass with some degree of regularity alternately to the right and left sides of the cord, but whether they at first turn to the right or left, they all eventually come to lie near the middle line, and an approximately equal number of giant fibres is found on each side of the middle line of the cord. For instance, the section shown on Plate 33, fig. 15, is from a specimen with eighteen giant cells, and is taken at the anterior end of the ninth segment (see fig. G, p. 450). The processes of the first two cells are very slender and soon disappear, one of them (II) in the second segment, and the other (I) in the fourth segment (see figs. 5, 7). The giant fibre from the small lateral cell in the second segment (fig. G and fig. 5) is slender, and is not traceable beyond the end of the eighth segment. The process from the last giant cell is also slender and its sheath thin, and is not recognisable except for a short distance after its exit from the cell (fig. 14). The processes of the remaining fourteen giant cells are seen in fig. 15; they run, at any rate for a considerable distance, in two groups—those of the cells numbered III, IV, V, VI, VIII, IX, X, and XI are situated in the dorsal region of the cord, and lie almost symmetrically with

regard to the middle line, while those from the remaining six giant cells, numbered XII to XVII, are found in the middle or mid-ventral region of the fibrous part of the cord, three on each side of the middle line.

The giant fibres do not run down the nerve cord perfectly parallel to each other, but, at frequent intervals, approach and cross one another, and sometimes intertwine, often increasing or decreasing in diameter in a way which renders the following of their course in sections a matter of some difficulty. There is, however, comparatively little intertwining of the giant fibres in the first ten or twelve segments. The giant fibre is sometimes flattened and almost band-like as it courses across the cord shortly after its exit from the giant cell, but on attaining its final position in the dorsal region of the cord it usually presents an oval or circular section, which is generally distinctly greater in diameter than sections of the same fibre in the earlier part of its course (*cf.* the sections of the same fibres in figs. 14 and 15, and in some of the preceding figures). Later, the giant fibres decrease slightly in diameter as they pass backwards. They run along practically the whole length of the nerve cord. Some of the larger ones, from two to six in different specimens, may reach to within one or two millimetres of the posterior end of the worm, the others have successively disappeared after running various distances down the cord. The giant fibres generally taper and disappear among the ordinary nerve fibres, but those which terminate in the last one or two millimetres of the worm sometimes end rather abruptly—that is, they do not gradually taper and become lost among the ordinary nerve fibres, but, on the contrary, dilate, sometimes considerably, at their ends.

The giant fibres of *Halla parthenopeia* are generally round or oval in transverse section, and their lumina vary in diameter from about 8 to 40 μ. Those from the smaller anterior and posterior cells are of less calibre than those from the larger cells which form the second, third, and fourth segmental couples, but, as remarked above, the diameter of the same fibre is by no means constant.

VII. Branches of the Giant Fibres.

Each giant fibre, as it traverses the course above described, gives off a number of branches. Soon after leaving the giant cell, as the fibre curves in the lateral region of the neuropile, a branch issues into the latter, rapidly tapers or ramifies, and is soon lost to view among the ordinary fibrous elements (figs. 3, 4, 5, 6). While often present, this branch could not be found in all the cases examined. As already mentioned, the axones of the anterior six to ten giant cells soon come to lie in the dorsal part of the nerve cord. Just before attaining this position the giant fibre begins to give off branches which generally issue from it ventrally and pass into the fibrous part of the cord (Plate 34, figs. 27, 28). Each branch consists of a protoplasmic axis like that of the giant fibre, but often slightly more deeply stained, surrounded by a sheath of the same nature as that of the giant fibre. Most, if not all, of the branches, which are at first 3 to 6 μ thick, almost immediately divide into

two, one twig passing to the right and the other to the left. These twigs gradually, or rapidly, taper and their sheath becomes much thinner, so that the tracing of their course in sections becomes a matter of great difficulty. Many of them have, however, been traced across the cord, a distance of 0·2 to 0·3 mm., into the lateral or ventro-lateral region of the fibrous portion, and in some cases up to a point not far from the border of the cellular part of the cord. Here the sheath of the twig usually disappears, and it becomes impossible to further follow the protoplasmic axial content, which is here a strand of only about 1 μ in thickness. The actual mode of ending of the protoplasmic axis of the twig remains undetermined, but in no case could it be traced into, or even to the base of, a spinal nerve. I am inclined to believe that the twig, as such, terminates at or near the point where its sheath disappears, and that its contained fibrillæ (see fig. 29) become free in the neuropile of the cord. Similar branches are given off from the giant fibres, from time to time, as they run along the dorsal region of the nerve cord. I have planned out the branches of these dorsally situated giant fibres in order to ascertain if they have a metameric arrangement. The plan shows that branches are not constantly being given off along the whole length of the giant fibre, but that several issue, either from one or from two or three different giant fibres, within a distance of about 0·1 to 0·15 mm., and then there is an interval of about 0·5 mm. before the next group of branches is met with. It is therefore evident that the branches are present in segmental clusters. Branches are usually given off from the giant fibres which lie on both sides of the median plane, but there is considerable difference, even in successive segments, in regard to the number of branches present.

In one of the worms in which the branches are most clearly shown it was found that of the nine dorsally situated giant fibres which were traced through eight successive segments only four give off branches, the other five run through these segments apparently without sending out any branches. This seems to indicate that there may be some localisation in regard to the distribution of the branches of the giant fibres, but the method of serial sections is not suitable for the investigation of this problem, the solution of which can only be attempted when a topographical staining method is available.

The giant fibres from the last six or eight giant cells run, for a considerable distance at any rate, nearly in the middle of the fibrous part of the nerve cord. Branches issue from these and pass into the fibrous mass, where they are usually soon lost to view (fig. 28, Br.*).

In two cases a giant fibre gives off a stout branch which runs longitudinally through several segments and behaves apparently just like the main trunk of the fibre. Such a condition seems best described as a forking of the giant fibre, for the branch is almost as large as the main trunk. In one of these cases the branch, immediately on issuing from the main trunk, crosses directly to the opposite side of the nerve cord and then runs posteriorly through several segments (fig. 15).

Giant fibres have never been found in *Halla* running forwards in the œsophageal connectives or into the brain as they do in several Polychætes and Oligochætes (see p. 489).

VIII. The Sheath of the Giant Cells and Giant Fibres of *Halla*.

Each giant cell is enveloped in a thick fibrous glia sheath which is continued, but in a diminished amount, along the whole extent of the giant fibre. The elements of the sheath have a concentric appearance, as seen in section, and nuclei are present at intervals (figs. 16, 23, 24, 47–50). Occasionally one or more of the inner glia nuclei project considerably into the cytoplasm of the giant cell or the giant fibre (figs. 24, 27, 28). In surface view and in maceration preparations the interlacing glia fibrils which compose the sheath of the cell are seen to be elongate and thread-like, and reach, in many cases, a length of 50 μ. They are about 1 μ in thickness, but vary somewhat in this respect, though they are of more uniform diameter than those of the sheath of the giant fibre. The glia fibres lie in a granular matrix—the residual protoplasm of the glia cells.

In practically all the giant cells there are processes which run from the sheath into the cytoplasm. In most cells a comparatively few of the ingrowths are moderately stout and obvious by reason of their dark staining, and some, at least, of their components—namely, certain long fibrils—are clearly traceable almost to the nucleus. The other ingrowths are short and slender but very numerous, and are present over almost the whole peripheral region of the cell. Many of the larger ingrowths are obviously of compound nature at their origin and for the first part of their course, for they contain, besides one or more clearly staining fibrils, a matrix of glia protoplasm which is readily differentiated by its staining reactions from that of the giant cell into which it enters. In preparations stained with iron-hæmatoxylin the glia matrix stains more darkly than the peripheral protoplasm of the giant cell, in sections stained with toluidin blue and erythrosin it stains bright red, and in both cases its granules are well seen. Occasionally the nucleus of a glia cell is present at the base of these ingrowths. But the undoubted glia elements penetrate only a short distance into the cytoplasm of the giant cell, while the long fibrils which have entered through the sheath of the giant cell are seen running inwards and are, in many cases, apparently continuous with the network of intracellular neurofibrillæ presently to be described (pp. 477–479). The nature of these ingrowing fibrillæ may be more conveniently discussed after the intracellular network has been described (see p. 481).

The sheath of the giant fibre is abundantly provided with long, deeply-staining, often interlacing glia fibrils of different diameters. The thicker fibrils run chiefly longitudinally and in the outer part of the sheath, but in the inner part there are many thinner ones which are closely interlaced and form a fine meshwork ensheathing the nervous elements (figs. 37, 38). The fibrils are embedded in the finely granular

glia protoplasm. Nuclei are seen at intervals on or near both the thick and thin fibrils, and some of the nuclei project into the lumen of the giant fibre. Tags of the glia sheath projecting into the lumen of the giant fibre, such as are often seen in *Arenicola* and *Lumbricus*, are seldom met with in *Halla* or *Aglaurides*. The sheath of the giant fibre in *Halla* is not blackened on treatment with osmic acid, it is stained brown; it differs in this respect from that of several other Chætopoda. SCHULTZE (1879, p. 106) was apparently the first to definitely record the blackening of the sheath of the giant fibre, using that of *Lumbricus*, on treatment with osmic acid, an observation confirmed by VIGNAL (p. 384), FRIEDLÄNDER (1891, p. 247), and SCHNEIDER (1902, p. 399). LANGERHANS (1880, p. 91) first observed this blackening action of osmic acid on the sheath of the giant fibre of a Polychæte—*Prionospio*, and subsequently similar action of this acid on the corresponding structure in *Mastobranchus* was observed by FRIEDLÄNDER (pp. 223–225), and in *Clymene* and *Axiothea* by LEWIS (p. 232). Several of these authors have drawn attention to the fact that myelin or a myelin-like substance is present in the sheath of the giant fibres which they examined. But there can be little or none of this substance in the giant fibre sheath of *Halla* and *Arenicola*, for osmic acid has little darkening effect on the substance of the sheath in either of these cases.

IX. The Neurofibrillæ of the Giant Cells and Giant Fibres of *Halla*.

Methods.

After prolonged trial observations on various Annelids I decided that, of these, *Halla* afforded the best opportunities for the investigation of the giant cells and giant fibres. Preparations were made according to the method of GOLGI, but without the least success, a result identical with that obtained by VON LENHOSSÉK, RETZIUS (see p. 440), and, in fact, everyone who has attempted to investigate these elements by this method. Various methods of preservation and staining were tried in the hope of obtaining a clear differentiation of the elusive fibrillæ in these remarkable elements, but for a period of nearly five years without any really satisfactory result. I devoted much time while in Naples, in 1900 and again in 1906, to the *intra-vitam* staining of specimens of *Halla*, or portions of its nerve cord, with methylene blue, but this treatment proved totally ineffectual, although numerous modifications were made in regard to the strength of the solution employed and its mode of application. The staining fluid was in some cases injected into the living animal and allowed to act for varying periods before the nerve cord was examined; in other cases, portions of the nerve cord were dissected out and placed in solutions of methylene blue of various strengths, but with the invariable result that the giant cells and fibres were either unaffected or small portions of the giant fibres were stained in a diffuse and quite unsatisfactory manner, the small amount of stain absorbed being apparently only in the sheath. I do not know that any worker on Polychæta has been able to obtain, by this method, any insight into the structure of giant cells

or giant fibres. RETZIUS (1891) was able only exceptionally to obtain staining, and even that diffuse, of portions of the giant fibres of *Nephthys*, but those of *Nereis* and *Arenicola* and the giant cells of all three worms were totally unresponsive. HAMAKER found the method of some service for the study of the topography of the giant fibres of *Nereis*. The same method has yielded only moderate results when applied to the corresponding structures in earthworms. Although CERFONTAINE succeeded in obtaining with methylene blue a general staining of the giant fibres of *Lumbricus* which enabled him to more readily trace their course and to observe their branches, this method of staining did not serve to bring into view any fibrillar structures in the giant fibres, nor did it even indicate the presence of giant cells; these latter remained totally indifferent to the stain, so that CERFONTAINE had to depend upon the method of serial sections for the demonstration of the connection between the giant cells and the giant fibres. KRAWANY (1905), by the use of methylene blue, was able to differentiate a single fibril in the giant fibre of an earthworm, but, so far as I know, this is the solitary success, in regard to fibrillar differentiation in the giant cells and fibres of Annelids, standing to the credit of methylene blue. This somewhat extended note on the unresponsiveness of these elements to the methods of GOLGI and EHRLICH will, I hope, anticipate any criticism that these methods have not received, both in my hands and those of others, a fair and even lengthy application to the giant cells and fibres of Annelids; they were only abandoned, as far as I was concerned, when it became quite clear that they were inefficient when applied to the giant nerve elements of *Halla*, *Arenicola*, and other Polychæta used in my experiments. Why these elements should be so refractory to impregnation with silver and with methylene blue I do not know; the density of the sheath, which prevents or retards the entry of the fluids used, may be a determining factor, but probably some chemical or physical peculiarity of the contents of the cells and fibres is also involved.

Other specimens were prepared according to APÁTHY'S gold method, but these did not yield a satisfactory result. APÁTHY'S Hæmatein 1A proved to be a serviceable stain for general purposes, but not for the sharp differentiation of the neurofibrillæ in the giant elements.

Attention was next given to the method of BETHE (1900) for the differentiation of neurofibrillæ, and specimens were preserved in 3-per-cent. nitric acid, and subsequently treated with ammoniated alcohol, acid alcohol, and ammonium molybdate. They were then imbedded and cut into sections, which were differentiated (as regards their contained ammonium molybdate), and stained on the slide with toluidin blue. In spite of much care and time expended upon these operations the results were not satisfactory. The modification of this method suggested by PRENTISS (1903) was then tried. Material fixed in sublimate was sectioned, and the sections molybdenated on the slide, differentiated, and stained with toluidin blue. This method yielded better results; in fact, the preparations so made showed the neurofibrillæ in some of the giant fibres, but they were much less

satisfactory in regard to the fibrillæ in the giant cells. It then occurred to me that possibly the material fixed and treated by BETHE'S method, in which, therefore, the affinity for stains of the cytoplasmic granules had been suppressed, might afford a better chance of obtaining differentiation of the neurofibrillæ on staining with iron-hæmatoxylin, and such proved to be the case. Preparations of this kind were then made in considerable number and very carefully differentiated, and they yielded the first clear views of the neurofibrillæ in the giant cells. In these preparations the cytoplasm is stained very pale and almost uniformly, and the slender fibrillæ stand out upon this pale background with considerable clearness (see, for instance, figs. 22, 23, which are drawn from preparations made by this method). Other specimens intended for the study of the cytoplasm and fibrillæ of the giant nerve elements were preserved in various other ways—in HERMANN'S fluid, FLEMMING'S mixture, formalin, etc.—but the best results were obtained from material fixed in sublimate-acetic mixture (saturated solution of corrosive sublimate 95 parts, glacial acetic acid 5 parts), and from that treated by BETHE'S method, as above noted. Material preserved in the sublimate-acetic mixture was carefully washed in alcohol (to which a little tincture of iodine had been added) until all trace of the mercury salt was removed. The anterior portion of the worm or of its nerve cord was then carefully dehydrated, imbedded, and cut into sections; those 8 μ thick were found to be most serviceable for the study of the neurofibrillæ, thinner ones do not permit the fibrillæ to be readily followed. The sections were stained on the slide with iron-hæmatoxylin, but great care is necessary in the differentiation if crisp definition of the fibrillæ is to be obtained. I regret that the details of CAJAL'S reduced silver method for exhibiting the neurofibrillæ in nerve cells and fibres only came into my hands after I had left Naples in 1906, so I could not give this method a trial there, but material preserved in formalin and in alcohol (with the addition of a small amount of ammonia in each case), according to the directions given by CAJAL, has been sent to me from Naples either in these mixtures or in silver nitrate solution, and has undergone subsequent treatment here. The sections of these specimens (a dozen in number) do not exhibit any neurofibrillæ in the giant cells or fibres. The first part of the giant fibre, that is, as it issues from the cell, often contains a single dark axial strand, but this is not a neurofibril, it represents the whole contents (axis cylinder) of the axone

The Neurofibrillæ of the Giant Cells of Halla (Plate 34, figs. 22–26).

The neurofibrillæ of the giant cells of Annelids have hitherto defied all attempts to render them clearly visible. GAMBLE and ASHWORTH (1900, pp. 487–489) observed, at the point of origin of the cell process of the giant cells of *Arenicola grubii*, certain fibrillæ, which they believed to be neurofibrillæ, but they could trace these only a very short distance into the peripheral part of the cell. This, and the similar account by the latter author (1904, p. 51), are apparently the only references

to the presence of neurofibrillæ in the giant cells of Annelids, and the fibrillæ shown in the preparations which these authors described, judging from the observations to be recorded below, probably form but a small portion of the neurofibrillæ actually present in the cells.

In many of the preparations of the giant cells of *Halla* which have been stained with iron-hæmatoxylin (see above, p. 477), slender fibrillæ may be seen forming a network throughout the whole or the greater part of the protoplasm of the cell. Objection may possibly be taken by some to the designation of this network as neurofibrillar, on the ground that the stain employed is not specific for neurofibrillæ but may also stain glia fibrils. Such objection would be answered by stating that the specific stains for neurofibrillæ have been inefficient when applied to these cells (see pp. 475, 476), but that the morphological relations of the stained network provide a good and safe criterion by which its nature may be decided. So judged, the network is undoubtedly of the same nature as the neurofibrillar network described by APÁTHY and others in the ganglion cells of various animals—especially of certain Annelids, for, as in these, so in the giant cells of *Halla*, the network is in perfect continuity with the fibrillæ of the axone (see below, p. 485). As there can be no doubt that the latter are neurofibrillæ* (APÁTHY), so also must the strands of the intracellular network be regarded as nervous fibrils.*

For the purposes of description this network may be divided into two portions: (1) a central portion usually situated in close proximity to, and enveloping, the nucleus; and (2) a more extensive wider-meshed and, generally, more slender-stranded network in the general cytoplasm. As already described (above, pp. 467–470), there is, immediately surrounding the nucleus, a zone of protoplasm, usually about 3 to 5 μ in breadth, which is more refringent and more deeply staining than the rest of the protoplasm; at the margin of this chromatic zone is found the central or perinuclear network of fibrillæ which takes the form of the surface of a sphere or an ovoid (figs. 53–56). In some giant cells the chromatic zone is enlarged, in which cases the perinuclear network is situated further from the nucleus; in other giant cells the specialised zone of protoplasm is diminished to a film only 1 or 2 μ thick, so that the central network of fibrillæ comes to lie very near the surface of the nuclear membrane. This perinuclear network consists of comparatively stout fibrillæ (fig. 25), about 0·2 to 0·3 μ in thickness, which form a close meshwork from which no fibrils pass centripetally, *i.e.*, towards the nucleus (fig. 22), but numerous fibrils are given off centrifugally and unite with the general network in the cell. This latter network usually consists of more slender fibrillæ, about 0·1 to 0·15 μ in thickness, arranged in wider meshes; it is continuous centrally with the perinuclear

* These are used as descriptive terms to indicate fibrillæ homologous with those described by APÁTHY, though it may be borne in mind that several authors have recently urged that the neurofibrillæ are either not conducting elements or are not the sole structures concerned in the conduction of the nerve waves (see, for instance, CAJAL, 1906).

net and peripherally with certain fibrillæ which enter the giant cell through its investing sheath (see below, p. 482). In a few cases the network in the various parts of the giant cell is more uniform both in the size of its meshes and in the thickness of its constituent fibrils, that is, the differentiation into a closer-meshed, more stoutly-stranded, perinuclear net, and a more open-meshed slender-stranded general network is less strongly marked, but is still generally recognisable, although the fibrillæ of the perinuclear net are almost as slender as those of the general network. This condition was noticed more frequently in the smaller anterior giant cells; in the other cells, situated further back in the same worm, the usual well-marked perinuclear network, composed of stouter strands, is present. As a rule, however, both in the anterior giant cells and in those situated further back in the series, there is a moderately sharp boundary between the compact perinuclear net and the looser general network of the cell, but in a few cases stout strands extend outwards from the perinuclear net and form a gradual transition between this and the general network.

These remarkable cells thus agree with many other nerve cells of Invertebrates, described by APÁTHY, BETHE, and others, in that they usually possess a specialised perinuclear network as well as a general or cortical network. CAJAL (1906, pp. 107, 108) has pointed out that in the majority of the ganglion cells of the leech a perinuclear network only is present, but as one ascends the animal series a cortical network is added which, in the nerve cells of many Vertebrates, becomes enormously developed. He has also shown that in many nerve cells which, when fully grown, possess a perinuclear and a general network, the perinuclear network is the first portion of the neurofibrillar network to be developed. He therefore regards the perinuclear network as primary both in phylogeny and ontogeny. The perinuclear network in the giant cells of *Halla* appears to be of great importance, if one may judge from its position and relations, for it is closely associated with the nucleus—the controlling and active centre of the cell, and it is placed so as to be most advantageously situated with regard to the substances which diffuse from the nucleus into the cytoplasm. This chromophilous substance, though present in the general cytoplasm, is nowhere found in such dense accumulations as in the perinuclear zone with which the perinuclear network is so intimately associated. It would appear, therefore, that this central network is specially situated with regard to the facility for rapid nutrition and for experiencing the full benefit of its close proximity to the nucleus. Some of the nerve elements described by BETHE seem to afford ground for accepting his view that the cell body in these cases may be regarded as being of subsidiary importance, as, in fact, a lateral, perhaps merely trophic, appendage of the conducting elements or fibres. But whatever may be the relative value of the cell body and its processes in the case of certain crustacean nerve cells, I think no one, after examining the giant cells of *Halla* and noting their large size and the high degree of development of the neurofibrillar network and especially of the perinuclear

network, could doubt that, in this case, the cell and its contained fibrillæ form a centre of activity which must exercise a profound effect upon the nature or potential of the nerve wave which traverses this centre. This statement must not, however, be taken as applying to the giant cells of all Annelids, for there are indications that the giant cells and fibres of certain worms provide one of the best examples of that disproportion in size, to which BETHE has drawn attention, between the small nerve cell and its process on the one hand, and the stout fibre into which the process enters, on the other. In such cases it would appear that the cell body is of subsidiary importance.

On passing to the consideration of the other fibrillar elements of the giant cells of *Halla* we are at once brought face to face with a most difficult question, namely, the nature of the fibrils, which are present not only in these giant cells, but which have been observed by several investigators in other nerve cells, leaving the sheath of the cell and penetrating more or less deeply into the cytoplasm. Such fibrils have been previously described in the nerve cells of Annelids, Crustacea, Mollusca, and various Vertebrates. For instance,* ROHDE (1887, p. 28) described and figured (fig. 36) large ganglion cells in *Sthenelais* at the periphery of which he observed non-granular fibrils which on the one hand run into the connective tissue sheath of the cell and on the other gradually merge into the fibrils on which the cell granules are arranged. In 1895 (pp. 387, 388, fig. A) the same observer described coarse neuroglia fibres extending into the ganglion cells of *Helix*. As the result of similar observations on the nerve cells of various Chætopoda, WAWRZIK (p. 123) concluded that the subcuticular fibrous tissue not only envelopes the nerve elements but penetrates into their interior, becoming merged in the spongioplasm. APÁTHY (1897, p. 603) described, in the ganglion cells of *Hirudo* and *Aulastoma*, outer and inner glia zones characterised by the presence of glia fibrils; in the latter of these leeches radial ingrowths ("Balken") pass from the inner glia zone into the somatoplasm of the ganglion cell and there become resolved into fine fibrillæ. Similarly, GAMBLE and ASHWORTH (p. 487) observed fibrillæ from the sheath, interpreted as glia fibrils, penetrating into the protoplasm of the giant cells of *Arenicola grubii*, in most cases into the outer layer of protoplasm only, but in one or two cells extending further and forming a definite network. HALPERN (1903, pp. 437–439) describes, around the "giant cells" of *Astacus*, a fibrillar envelope from which fibrils enter the cell plasma, but only to a limited depth; while he considers that their course and staining reactions indicate their glial nature, he is doubtful whether they are comparable to the true glia fibres of Annelids. In the spinal ganglion cells of *Lophius*, stained with iron-hæmatoxylin, HOLMGREN (1899, p. 137) found deeply staining undulating

* It is not intended to give a complete summary of the lengthy literature dealing with the fibrillar structures entering nerve cells, but merely to cite a few typical instances, some of which bear on the question at issue. For a more complete survey of the literature, reference may be made to papers by HOLMGREN, BETHE, VON BERGEN, HELD, and others.

fibrils of varying length and thickness imbedded in the cell capsule or situated between the capsule and the cell protoplasm and connected together by means of other fibrils. In tangential sections the fibrils are seen forming a basket-like network around the cells. Occasionally strong fibrils, apparently from some other region of the ganglion, come into contact with a cell, break up into fine branches on its capsule and, from various points of the pericellular network so formed, fine fibrils enter the cell. He concluded that these fibrils are nervous, a view confirmed by subsequent further study of the preparations (1900, p. 53). In the latter paper he also describes darkly stained fibrils in the ganglion cells of *Palæmon* and *Hirudo* (pp. 56, 57) and points out that the unipolar cells of the latter are surrounded by a network, deeply stained with iron-hæmatoxylin, which at various points sends into the cell branches, which are probably connected with the perinuclear network (but see below, HOLMGREN, 1904). SIMON* (1896) had already described, in the nerve cells of the leech, stained *intra-vitam* with methylene blue, a loose fibrous network in the pericellular capsule from which fibrils pass inwards and merge into the intracellular network. According to HOLMGREN (1901, p. 293), the nerve cells of *Helix* are more or less penetrated by processes† of the adjacent glia tissue, which may branch richly in the nerve cell to form a network, in the strands† of which sinuses or canals may be present, communicating with similar canals outside the cell and forming a trophic mechanism (trophospongium). HOLMGREN, later (1904), concluded that, in *Hirudo* also, the fibrils which enter the ganglion cells from their sheaths are glia fibrils which form the trophospongium of these cells. CAJAL has recently described and figured (1905, fig. 9A, p. 96) a network of fibrillæ situated in the pericellular capsule of the cells of the sympathetic ganglia of man, from which fibrillæ—afferent nerve fibrils—pass inwards into the cytoplasm of the cell. Of these observations those by SIMON, HOLMGREN (1899, 1900), and CAJAL are of most interest in the present connection, for they seem to agree in several respects with the conditions existing in the giant cells of *Halla*.

When I first observed (in 1900) fibrils from the sheath extending into the cytoplasm of these cells I was inclined, following previous observers,‡ to look upon them all as glia fibrils, but later (in 1906) when, in the preparations which clearly showed the neurofibrillar network in the cells, it was seen that some of the fibrils from the sheath enter into close association with this network, it became questionable whether these fibrillæ could longer be regarded as glial. The difficulty of finally deciding this question must be obvious, especially to those who have had any experience of determining whether or not there be continuity of two such minute structures as these entrant fibrils and the strands of the intracellular network. If

* I have been able to see only an abstract of this paper.

† Which are of considerable breadth.

‡ HOLMGREN'S recently published paper (1900) and the notice of SIMON'S paper had not then come into my hands.

these fibrils are actually continuous with the neurofibrillar network they must, of course, be nervous, but it is quite conceivable that, if they are of glial nature, they may still come to lie in close association, without being continuous, with the neurofibrillar network. Frequent observations on many different giant cells show that some of the entrant fibrils may be traced inwards even to the perinuclear net, by which time they have subdivided into fibrillæ which are apparently identical and continuous with the strands of the neurofibrillar network (figs. 24, 25, ENT. F.). Such long, sharply stained fibrillæ seem, therefore, to be nervous rather than glial. There are, however, in many of the giant cells other fibrils which enter the cell in clusters, sometimes accompanied by a "sinus"; these correspond moderately closely to the glia fibrils described by various authors in other nerve cells, and may be so regarded in this case. They are usually shorter and less sharply stained than the fibrils mentioned just above (see fig. 31).

A few of the processes from the sheath which enter the giant cell are comparatively stout (see p. 474), and, as regards many of them, it is clear that, close to their point of origin, they are of compound nature, for a small amount of granular, probably glial, protoplasm invests their bases, that is, at the point where they leave the sheath to enter the protoplasm of the giant cell; but this granular investment soon ceases, and the deeply and sharply stained fibril runs on alone, extending well into the cytoplasm. In other cases fibrils enter the cytoplasm without any accompaniment whatever of glial protoplasm, and these, in particular, are often long, extending inwards as much as 40 μ, and most clearly show intimate association with the elements of the neurofibrillar network. Soon after entering the cytoplasm of the giant cell these fibrils divide or give off branches which, spreading, apparently in some cases become connected with a considerable area of the neurofibrillar network, and may, as already stated, be frequently traced even into the perinuclear network. It is difficult to avoid the conclusion that such fibrillæ are of the same nature as the intracellular network and therefore nervous, in which case they are probably afferent fibrils (assuming the axone fibrils, described on p. 485, to be efferent) and are equivalent to the dendrons or dendrites of other nerve cells.

In addition to the above described more obvious entrant fibrils there are usually very slender, short, and numerous fibrillar ingrowths extending from the sheath into the cytoplasm over the whole periphery of the giant cell (figs. 18, 21, 24, GL. F.). Their extreme slenderness renders them very difficult to stain satisfactorily, but nevertheless it can be established that many of the fibrillæ divide shortly after entering the cell, and their branches form, in some cases, a meshwork of interlacing strands situated a short distance from the periphery of the cell and well seen in a tangential section through this particular plane. The strands of this meshwork are, however, rather granular in places, and do not present the sharply stained appearance of the neurofibrillar network. These are glia fibrils, and do not generally penetrate beyond the clear peripheral zone of protoplasm, that is a distance of

about 5 μ. They are, in some cells, so numerous as to mark a definite zone of the protoplasm with radial striations, so producing, perhaps, an equivalent to one of the glia zones described by APÁTHY in the ganglion cells of the leech (see fig. 21).

I have not been able to ascertain the place of origin of the stouter, apparently nervous, fibrils which enter the giant cell through its sheath as described above (p. 482), but I can state that many of them appear to come from the direction of the fibrous part of the cord. They run upon or in the sheath of the giant cell for varying distances, and then pass through the sheath into the protoplasm of the cell. The only method of staining hitherto found practicable for bringing out the neurofibrillæ within and without the giant cells, namely, iron-hæmatoxylin, also stains the glia fibrils which are present around these cells in large numbers. Until some process is found by means of which the neurofibrillæ only are stained the definite settlement of the nature of the entrant fibrillæ, and, if they be nervous, their place of origin, will be extremely difficult if not impossible.

We may summarise the present position of this most difficult portion of the subject thus: There are fibrillæ, which are generally sharply and deeply stained, which, after penetrating the sheath of the giant cell, enter the cell, and are traceable a long way (40 μ) into the cytoplasm, even as far as the perinuclear net; the branches of these fibrils enter into close association and, in many cases, seem to be continuous with the intracellular network, and are, therefore, apparently neurofibrillæ. Numerous other fibrils also enter the cell from its sheath. These are very short (5 μ) and slender, and their branches form a meshwork in the peripheral zone of the protoplasm; their structure and relations suggest that they are glia fibrils. Somewhat longer fibrils of a similar nature are occasionally seen entering a giant cell accompanied by one or more sinuses (Saftkanälchen).

The Neurofibrillæ of the Giant Fibres of Halla.

The information available regarding the nature and minute structure of the giant fibres of Annelids is of a very contradictory description. As pointed out in the historical section of this paper (pp. 429–446), the contents of these remarkable fibres have been regarded as of truly nervous nature, as degenerate nerve substance, and as non-nervous; in the last case, either no function was ascribed to the substance or it was regarded as supporting or as nutrient material. That these fibres are true nerve fibres may now be regarded as firmly established, but on reading the accounts of those by whom this view is accepted and advocated a wide divergence of opinion is met with regarding the structure of the contents of the fibres. LEYDIG (1864, p. 155) described the contents of those of *Lumbricus* as a pale band which, he concluded, corresponds to the axis cylinder of a vertebrate nerve fibre. According to H. SCHULTZE (1879, p. 106) and KULAGIN (p. 404) a central bundle of fibrils is

present in these fibres of the earthworm, and SPENGEL (pp. 39, 40) observed a faint longitudinal striation in those of *Lumbriconereis* and *Spirographis*, but VIGNAL (p. 385) could not find the least trace of striation in the contents of the earthworm's giant fibres. ROHDE (1887, p. 53) noticed fibrils passing from the "axis cylinder" in the centre of the giant fibre of *Sthenelais* across the lumen of the fibre and into its sheath, and suggested that they pass into the central substance [that is, the fibrous part] of the cord. NANSEN (1887A) considered the giant fibres of *Lumbricus* (and *Nereis*) to be composed of a number of closely apposed, minute, primitive tubes, but FRIEDLÄNDER (1888 and 1891) was unable to ascertain any definite striation in the watery contents of these tubes, and HALLER (1889) considered their contents to have a reticulate structure. According to WAWRZIK (pp. 121, 122), the neural canals, both of *Lumbricus* and *Myxicola*, contain, like the nerve cells, a fibrillar spongioplasm and a homogeneous fluid hyaloplasm, the fibrillæ of the former being continuous with those of the sheath of the fibre and therefore of the nature of supporting, and not essentially nervous, structures. LEWIS (p. 233), who made a careful study of the giant fibres of *Clymene* and *Axiothea*, found their contents to be uniform throughout, nothing of a fibrillar or striate nature being discoverable. HAMAKER found that, in sections of preserved specimens of *Nereis*, the contents of the giant fibres were marked out into polygonal areas, but this is surely an artefact. CERFONTAINE'S figures (figs. 5, 6, 7, 8) of his methylene-blue preparations do not exhibit any fibrillar structure of the contents of the giant fibres of the earthworm. GAMBLE and ASHWORTH (fig. 66) figure fibrils in a giant fibre of *Arenicola*, but, in the light of subsequent experience, these are of doubtful character. This review of the statements concerning the structure of the giant fibres shows that the various authors concerned had seen in their contents either no structure at all or they had noticed only faint or vague striæ or fibrillæ or reticulate artefacts.

APÁTHY (1897, pp. 574, 624) first demonstrated true morphological elements—the neurofibrillæ—in the giant fibres ("sensorische Schläuche"), using those of the earthworm. He described the fibrillæ as sinuous, very thin "primitive fibrillæ," among which one to three thicker fibrils are often found, a description entirely confirmed by SCHNEIDER (1902, p. 403). JOSEPH'S section (1902, fig. 26) of the giant fibres of the earthworm shows one central fibril in each giant fibre, and CAJAL (1904, p. 285) and KRAWANY (p. 303) also describe and figure only a single fibril in each giant fibre. It is curious that of the five observers who have described neurofibrillæ in the giant fibres of the earthworm—the only Annelid in which they have hitherto been clearly seen—two state that several fibrils are present in each giant fibre, while three find only a single fibril. There are three possible explanations of these divergent results: (1) the processes used by CAJAL, KRAWANY, and JOSEPH may have caused fibrils previously separate to cohere, and so appear as a single fibril, as in the case of one of the specimens of *Halla* described below (p. 486); (2) the variations may be due to the different functional conditions of the specimens used:

for example, some of the worms may have been taken in summer and others in winter, and consequently show morphological variations in their neurofibrillæ, corresponding to different functional activities, similar to the changes described by CAJAL (1904A) in the neurofibrillæ of active and starved leeches; (3) different species or even genera, of earthworms may have been used in which the neurofibrillar conditions are not identical.

It is worthy of note that in no case thus far recorded have fibrillæ from giant cells been traced into and along the giant fibres.

The origin of the fibrillæ of the axone or giant fibre can be clearly seen in the giant cells of *Halla*. From the fibrillar network in the cell protoplasm (see p. 478) neurofibrillæ pass into the cone of origin of the axone. These fibrillæ are at first very slender, sometimes more slender than many of those of the general network in the cell, but, as they pass further away from the nucleus and towards the axone, fibrils distinctly stouter than those of the general intracellular network are formed, each due to the fusion of several of the slender fibrillæ. These thicker fibrils, so produced, may be traced into and along the giant fibre (figs. 22, 23). These fibrils of the axone do not appear to be specially associated in their origin with the perinuclear network; they are usually formed by the union of slender fibrils from the general (that is, more peripheral) network, but in a few cases—for instance, in some of the small cells in which the central network extends nearly to the cone of origin of the axone—the axone fibrillæ arise, in part at least, from the perinuclear network. The small cells are more favourable for the study of the connections of the neurofibrillæ of the axone with the intracellular network, and this for two reasons: firstly, because in these cells the network is usually rather less complex than in large cells; and, secondly, because a central section through the axone, including its fibrillæ, also contains a greater proportion of the intracellular network than is the case in a section of the same thickness through a large cell, and therefore the connections of the axone fibrillæ with this network are more likely to be visible. These connections are clearly seen in figs. 22, 23, which are drawn from a small anterior giant cell; in fact, from the first, or most anterior, giant cell of the specimen. In some of the giant cells, especially in the larger ones, the axone fibrillæ arise from a series of very fine fibrils which issue from the general network. The extreme tenuity of these fibrils greatly increases the difficulty of establishing the connection between the intracellular network and the axone fibrillæ.

The number of fibrillæ issuing from the cell varies and is correlated, to some extent, with the size of the cell, and consequently of the giant fibre in which the fibrillæ lie. From the small anterior giant cells only a few fibrillæ, up to about half a dozen or, in some cases, ten, may be counted, and these form a loose bundle 2 to 3 μ in diameter, lying in a giant fibre, the internal diameter of which is 7 to 10 μ (fig. 39). In one case—a very small anterior giant cell—only a single fibril could be seen entering the giant fibre. In the larger giant fibres a considerably greater number of

fibrillæ is present, but, owing to their contiguity, it is very difficult to count them, but from a dozen to twenty, or occasionally nearly thirty in the largest fibres, may be given as a moderately reliable estimate of their number. In some of my preparations these fibrillæ are loosely arranged and occupy the greater portion of the lumen of the giant fibre; for instance, the bundle of neurofibrillæ is 15 μ in diameter and the internal diameter of the giant fibre in which it lies is 20 μ. In other preparations the neurofibrillæ lie in a more compact bundle in the middle portion of the giant fibre. In one or two specimens the fibrillæ in the giant fibres are so closely applied to each other that it is only possible here and there to distinguish them individually; that is, the fibrillæ and their interfibrillar substance have become cemented together to form one strand from 2 to 3 μ in diameter. But this last condition is, I think, attributable to the too rapid withdrawal, during preservation, of water from the interfibrillar substance, producing its sudden contraction, in which condition it became fixed. As a rule, the delicate, darkly staining fibrillæ are separately distinguishable and lie more or less parallel to each other (figs. 39, 40). Judging from my best preparations, the bundle of neurofibrillæ probably does not fill the giant fibre in life, but occupies, in different cases, from about one-fourth to three-fourths of the internal diameter of the fibre, the remaining space being filled with the semi-fluid perifibrillar substance (see below, p. 487).

I have never succeeded in seeing the fibrils in the living or fresh giant fibres.

The fibrillæ in a giant fibre are usually all of the same thickness, but in several fibres one to three fibrils thicker than the rest are seen. These thicker strands are not due to *post-mortem* association of two or more fibrils, for, on tracing each of them backwards into the cell from which it arises, it is seen that, from its origin or shortly afterwards, the fibril is rather stouter than the neighbouring axone fibrillæ, and owes its greater thickness either to its origin from a portion of the intracellular network composed of thicker strands or to a fusion of a larger number of primitive fibrils than usual (fig. 22). Such thicker axone fibrils are traceable far into the giant cell, while the slenderer ones are difficult to follow into the cell much beyond the cone of origin of the axone. Similar thicker fibrils and their relations to the intracellular network are well seen in *Aglaurides* (p. 497). Apáthy and Schneider observed among the thinner fibrils in the giant fibres of *Lumbricus* a number (one to three) of stouter ones, and a similar condition of the fibrillæ is described in the "sensorische Schläuche" of *Hirudo* by Apáthy (p. 559) and Bethe (1903, p. 36). From the explanation given above, the greater thickness of one or more fibrils in the giant fibres of *Halla* may be regarded as a circumstance associated with their origin from thicker or more numerous strands of the intracellular network. These thicker fibrils have not, so far as can be seen, any special significance, for they have only been met with in a small proportion of the giant fibres, which were not distinguished in any other way from those in which the fibrillæ are of equal thickness.

The neurofibrillæ of the giant fibres of *Halla*, measured immediately after leaving

the giant cells, usually have a thickness of about 0·2 to 0·25 μ, but they are distinctly thinner further away from the cell.

On examining longitudinal sections of the nerve cord, the neurofibrillæ are seen running along the whole length of the giant fibres. In most cases, owing to the contraction of the worm on killing, the giant fibres and their contained fibrillæ are not straight, but have a sinuous or undulating course (fig. 40); in worms killed fully extended they are practically straight (fig. 39).

In life, the space between the bundle of fibrillæ and the sheath (for the structure of which see p. 474) of the giant fibre is filled with the semi-fluid perifibrillar substance, which is colourless, hyaline, and contains very fine granules. In the sections of preserved specimens the coagulated remains of this substance are very lightly stained, and present an alveolar or foam-like appearance, of course an artefact, in the strands of which the fine granules are seen (fig. 39). The neurofibrillæ are imbedded in a matrix—the interfibrillar substance—which seems to be somewhat more homogeneous than the perifibrillar material. The contents of the giant fibre are the equivalent of the axis cylinder of an ordinary nerve fibre, being a direct continuation of the protoplasm of the giant cell. The perifibrillar and interfibrillar substance are merely portions, which it is convenient to designate by APÁTHY'S terms, of the protoplasm of this axis cylinder. In these giant fibres, as in many others, the axis cylinder is, in life, of a semi-fluid nature, and requires very careful treatment during preservation, otherwise the giant fibres appear to be almost, if not quite, empty. The substance of the axis cylinder, apart from the contained neurofibrillæ, is remarkable for the small amount of coagulable and stainable material which it contains. The strong sheath of the giant fibre is probably correlated with the semi-fluid and yielding character of the axis cylinder (see p. 445).

The structure of the contents of the giant fibres of *Halla* is very similar to that of the axis cylinder of a medullated nerve fibre of a Vertebrate as described by MONCKBERG and BETHE (1899), except that in the former there is nothing comparable to the Ranvier's nodes of the latter. The original view of LEYDIG thus receives full and striking confirmation, namely, "dass diese mittlere Substanz dem Achsencylinder entspricht . . . kann nicht in Zweifel gezogen werden" (1864, p. 155).

The branches of the giant fibre, the arrangement and general structure of which are described on pp. 472, 473, are extremely difficult to investigate with regard to their fibrillar contents, owing to the fact that their course is so seldom straight, but is curved or sinuous, so that only rarely is a good view of a branch obtainable for a sufficient length to permit the very critical observations required. Consequently all that I am able to state is that in several cases (but in comparatively very few considering the thousands of sections examined) one or a few (generally the latter) extremely slender fibrils were seen in the protoplasmic axis of these branches (fig. 29). As already explained (p. 473), this protoplasmic axis is traceable to the ventral or ventro-lateral region of the fibrous part of the cord, where it becomes lost to view.

Branches of the giant fibres could not be traced into the spinal nerves, and I am inclined to believe that they do not pass into the nerves, but end in the neuropile, probably in a similar manner to the branches of the "sensorische Schläuche" of *Hirudo* described by APÁTHY (pp. 561, 562) and BETHE, that is, the protoplasmic investment of the fibrillæ ends as above described, and the free fibrillæ then enter singly into the neuropile (*cf.* BETHE, 1903, p. 36, fig. 13). This, however, could not be definitely established in the case of the endings of the branches of the giant fibres of *Halla*.

X.—The Posterior Giant Cells and Giant Fibres of *Halla*.

Besides the giant cells in the first few chætigerous segments, which have been described above, there are, in the last few segments of *Halla*, from six to eight giant cells situated in the ventro-lateral region of the nerve cord. These giant cells are much smaller than the anterior ones, their mean diameter is from 25 to 30 μ. On account of the thinness of the sheath of these posterior giant cells and their corresponding giant fibres, the course of the latter is traced only with some difficulty, but in successful preparations it may be established that these giant fibres, immediately after issuing from their respective giant cells, cross to the opposite side of the cord and run anteriorly in the lateral regions of the neuropile. They may be traced forwards as far as the ninth or tenth segment, that is, almost into the region of the anterior giant cells. The minute structure of these giant cells and the arrangement of the neurofibrillæ in them and in their slender giant fibres will be described in a future portion of this work.

XI.—General Remarks on the Giant Fibres of Annelids.*

Two Types of Giant Fibres.

The giant fibres of Annelids are divisible into two distinct types which may be designated respectively as simple and compound according as each fibre is connected with one or with several giant cells. The former type is well seen in *Halla* (and *Aglaurides*), in which each giant fibre is an outgrowth from one giant cell and does not fuse or become merged with a similar outgrowth from any other giant cell, a statement which applies to both the anterior and to the posterior series of giant fibres. Similar simple giant fibres are found in *Sthenelais* (ROHDE, 1887) arising from the cells in the subœsophageal ganglion, but the fibres from the two giant cells in the brain of this worm are stated to fuse together to form a single (compound) giant fibre, and fusion of the giant fibres from the posterior giant cells is also stated to occur, but the evidence for this last assertion is not conclusive. It is interesting to note that the simple giant fibres of *Halla* and of *Sthenelais*, after leaving their respective giant cells, invariably cross the cord to the opposite side; this decussation

* For an account of the structure of the giant fibres of Annelids, see pp. 483, 484.

is quite constant both in the fibres from the anterior and in those from the posterior giant cells. The giant fibres of *Arenicola*, *Axiothea*, *Clymene*, and *Lumbricus* are of the compound type. In each of these worms the giant fibre, at first an outgrowth from a single giant cell, receives at intervals (segmental in *Arenicola*, irregular in the other worms named) processes from other similar giant cells which fuse completely with the original or primary giant fibre. These are the only cases in which the cellular connections of the giant fibres have been fully worked out.

The Course of the Giant Fibres.

The course of the fibres from the anterior giant cells of *Halla* and of those arising from the giant cells in the subœsophageal ganglion of *Sthenelais* is very similar, that is, the fibres decussate and run backwards, dorsal to or imbedded in the fibrous part of the cord, some of them almost to the posterior end of the animal. The fibres from the posterior giant cells in both these worms decussate and run forwards either in the dorsal or lateral portions of the fibrous part of the cord. These are the only simple giant fibres whose course has been fully investigated.

The compound giant fibres of *Arenicola*, *Clymene*, *Axiothea*, and *Lumbricus* to the number of one, two or three, are found near the dorsal side of the nerve cord and are present along the whole or the greater part of its length. We know too little of the internal arrangements of their fibrillæ (and nothing of the cellular connections of the latter) to be able to compare in detail these giant fibres with those of *Halla*, so that we cannot at present state whether these compound giant fibres are to be regarded as equivalent to all the giant fibres—that is, those from the anterior and those from the posterior giant cells—of *Halla* fused together. When more than one compound giant fibre is present there generally occur anastomoses between the various fibres, often situated at segmental intervals.

The giant fibres in other Chætopoda are usually dorsally situated, but in some cases they are found in the middle portion or at the sides of the nerve cord or even on its ventral surface (as in *Eunice* and *Magelona*). While the giant fibres in many Chætopoda are restricted to the ventral nerve cord it is worthy of note that in several they extend forwards through the œsophageal connectives into the cerebral ganglia; this is the case in *Spirographis*, *Prionospio*, *Sthenelais*, *Polynoe*, *Sigalion*, *Branchiomma*, *Sabella* and *Nereis virens* among Polychæta and in *Lumbriculus* and *Rhynchelmis* among Oligochæta. In two of these cases only have giant cells been described as occurring in the brain, namely, *Sthenelais* and *Sigalion*. In many cases the single compound giant fibre present in the anterior part of the nerve cord of Oligochætes forks in front, the two branches pass into the œsophageal connectives for a short distance and there terminate in fine-pointed ends.

The "sensorische Schläuche" of *Hirudo*, as described by APÁTHY and BETHE, are similar in their fibrillar contents to the giant fibres of *Halla*, *Aglaurides*, and *Lumbricus*, but nothing is known of the cellular connections of these "Schläuche."

Branches of the Giant Fibres.

When they were first discovered, the giant fibres were believed to be unbranched (CLAPARÈDE, 1862, p. 225; LEYDIG, 1864, p. 155), but later observers have found branches in many of the cases investigated. In some Annelids these branches are segmentally arranged, as was first noticed by EMERY (p. 397) in *Nephthys* and afterwards by HALLER (1889, p. 225) in *Lepidasthenia* and as described in the present paper (p. 473) in *Halla*, but the metamerism of the branches does not appear to be so clearly marked in other Polychætes. HALLER states that he followed segmental branches of the lateral giant fibre into the epidermal layer beneath the lateral muscle bundle. His fig. 8 is noteworthy as being the only figure, to my knowledge, which shows a branch of a longitudinal giant fibre running into a peripheral nerve of an Annelid. SPENGEL (p. 39) observed branches of the giant fibres of *Lumbriconereis* passing into the fibrous part of the cord, through which they could be followed almost to the ventral group of ganglion cells. GAMBLE and ASHWORTH (1900, pp. 490, 491) described branches of the giant fibres of *Arenicola*, some of which are stated to run down the sides of the nerve cord to the roots of the spinal nerves. In regard to Oligochætes—CERFONTAINE first pointed out the segmental arrangement of the branches of the giant fibres in the earthworm, in which these branches either subdivide and terminate in the ganglionic chain or pass out towards the periphery "comme fibres motrices." FRIEDLÄNDER (1894A, p. 668) also found branches issuing at segmental intervals from the giant fibres of *Lumbricus* and dividing into two twigs, one passing to the right and the other to the left. Similar branches were also noticed by VIGNAL in *Lumbricus*, by MICHAELSEN in *Pachydrilus*, and by MICHEL in *Allolobophora*, the branches in each case being lost to view in the fibrous part of the cord.

APÁTHY (p. 625) first noticed fibrillæ issuing from the giant fibres of *Lumbricus* and passing into the fibrous part of the cord. SCHNEIDER (p. 403) made the important observation that not only do the giant fibres of *Eisenia* (*Lumbricus*) *rosea* give off branches in which run neurofibrillæ which are soon lost in the fibrous part of the cord, but that, in addition to these, there are single fibrillæ which pass out from the giant fibre, perforating its sheath, and enter the neuropile. It is possible that this latter observation may explain why in certain cases no obvious branching of the giant fibres could be observed; for instance, LEWIS (p. 234) concluded, after careful examination, that branching of the giant fibres of *Axiothea* and *Clymene* does not occur. It is possible in these cases that, although massive branches are not given off, fibrillæ may issue singly or in very small bundles through the sheath into the neuropile, but the methods of staining employed did not serve to bring these fibrillæ into view.

In no case so far described have the giant fibres, or their branches or fibrillæ, been traced into association or communication with nerve cells other than the giant cells,

but APÁTHY and SCHNEIDER traced fibrillæ from the giant fibres into the neuropile of earthworms, and, as shown above (p. 473), the fibrillæ in the branches of the giant fibres of *Halla* also, almost certainly, become free from their protoplasmic investment and spread in the neuropile. The only other case in which giant fibres have been found entering into close association with other nerve elements is that of *Nereis virens*, described by HAMAKER, in which the giant fibres are pierced by another set of nerve fibres, which, HAMAKER considers, act as branches of the giant fibres and serve to put the latter "in relation with every segment of the body."

4. THE GIANT NERVE CELLS AND GIANT NERVE FIBRES OF *AGLAURIDES*.

Examination of sections of eight specimens of *Aglaurides fulgida** serves to confirm the main results obtained from the study of the giant cells and fibres of *Halla*. As in *Halla*, there are two series of giant cells, an anterior and a posterior, but only the former is dealt with in this communication; the posterior series is briefly noticed on p. 498.

I.—THE NUMBER AND ARRANGEMENT OF THE GIANT CELLS OF *Aglaurides*.

The giant nerve cells of *Aglaurides* are segmentally arranged, there being usually a couple in each of the first few chætigerous segments, though occasionally† only a single cell is present instead of a couple. The two cells of each couple are usually found one behind the other, in a position corresponding to that of the segmental couples of *Halla* (p. 447), that is, in or near the mid-ventral line of the nerve cord, and so placed that the posterior one is close to the hinder border of the segment to which they belong, but they exhibit a greater tendency than in *Halla* to lie side by side; in several cases the two cells of a couple are found either in the same transverse plane or one of the cells is only very slightly behind the other (see figs. K, L, M).

In the youngest specimens of *Aglaurides* in my possession, 35 mm. and 45 mm. long,‡ there are already four segmental couples of well-developed giant cells (fig. J); in each of five other specimens, from 50 to 200 mm. long, there are five couples,§ or four couples and a single cell (figs. K, L); and in the largest specimen, 315 mm. in length, there are five couples and a single cell (fig. M). The giant cells of *Aglaurides* are almost invariably situated in the mid-ventral group of cells, only two out of the seventy-four cells examined were found on the right or left lateral group of cells. In both these cases, which occur in different specimens, these laterally situated cells are

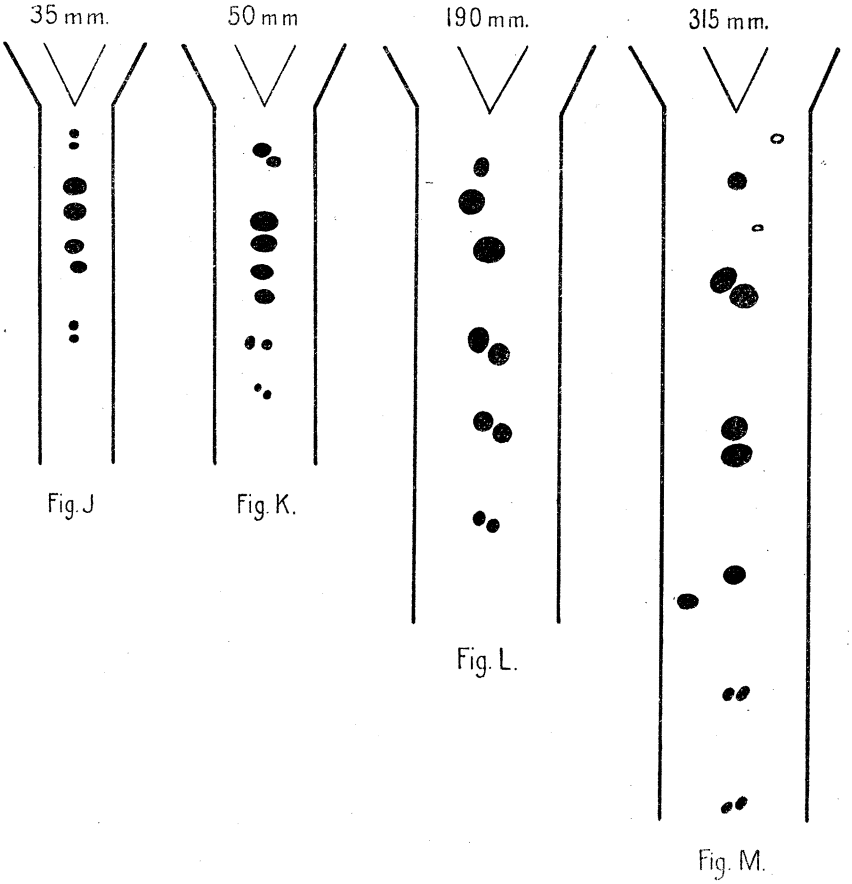
* See footnote, p. 428.

† In 10 per cent. of the segments examined in which giant cells occur.

‡ These specimens have 165 and 190 segments respectively.

§ Except in one case, in which a single small giant cell has apparently just been differentiated in the sixth segment.

primary and not secondary giant cells (see pp. 449, 451), for they are larger; in fact in one case the lateral cell is the largest cell in the specimen. In the same segment in which each of these occurs there is only a single giant cell in the median plane, with which the lateral cell closely corresponds in size (fig. M). As shown above (p. 451), such lateral cells are to be regarded as members of the couples of their respective segments, the couple in each case being composed of a lateral and a median giant cell.



FIGS. J, K, L, M.—Plans of the Nerve Cord in the first Six Segments of Specimens of *Aglaurides fulgida*, showing the relative position and size of the giant cells. The primary giant cells are drawn in solid black, the secondary giant cells (which are present only in one—the largest—specimen examined) are shown in outline. The length of the specimen, measured in the preserved condition, to which each cord belongs, is stated above each plan. In the specimen shown in fig. L there is a single giant cell in the first segment; the second and third cells which form the second couple are much farther apart than usual. Constructed from transverse sections. × 40.

In only one specimen have undoubted secondary giant cells been found. These are present in the anterior portion of the first and second chætigerous segments of the largest specimen (see below, p. 493).

The giant cells of *Aglaurides*, like those of *Halla*, make their appearance early, and the subsequent differentiation of further primary giant cells proceeds in a closely corresponding manner in both these worms. On comparing the number of giant cells

present in specimens of *Aglaurides* and *Halla* of the same length, it is found that there is always a slight advantage on the side of the latter, in which the formation of the giant cells is somewhat more accelerated in the early stages of growth of the worm, an advantage which is subsequently maintained. Young specimens of *Halla* up to 44 mm. in length have giant cells in the first five chætigerous segments (p. 447), while examples of *Aglaurides* 35 and 45 mm. in length possess giant cells only in the first four chætigerous segments. This difference is maintained henceforward, for in specimens of *Halla* 86 and 140 mm. in length giant cells have also been formed in the sixth and seventh segments, whereas in five specimens of *Aglaurides* 50, 70, 85, 190, and 200 mm. in length respectively giant cells are present only in the first five segments, except in one case where a single small giant cell has apparently just been differentiated in the sixth segment. The largest specimen of *Aglaurides*, 315 mm. in length, has primary giant cells in six segments; specimens of *Halla* 280, 310, and 350 mm. long have seven couples (or six couples and a single cell). In the rate of their differentiation the giant cells of *Aglaurides*, as it were, lag one segment behind those of specimens of *Halla* of the same length. But there is a much more marked difference with regard to secondary giant cells. As already stated, only two such cells have been met with in the eight specimens of *Aglaurides* examined. They occur in the first and second chætigerous segments of the largest specimen. Examples of *Halla* of the same length have three or four secondary giant cells, the two or three situated near the point of fusion of the œsophageal connectives and ventral nerve cord being especially constantly present (see pp. 449, 451). In only two specimens of *Aglaurides* have cells (one cell in each specimen) been found in or near the œsophageal connectives which are at all suggestive of secondary giant cells. Each of these cells is larger than the neighbouring nerve cells, but its cytoplasm and sheath do not agree in structure with those of giant cells, but are more like those of large ganglion cells. The occurrence of these cells in two comparatively small worms, 70 and 85 mm. in length respectively, is also against their interpretation as giant cells, for, had secondary giant cells already appeared in such young worms as these, well developed secondary giant cells ought to be found in the larger specimens, 135, 190, and 200 mm. in length, but examination of the œsophageal connectives and anterior region of the nerve cord shows that no such cells are present in these larger worms. We may therefore conclude that there are probably no secondary giant cells in the œsophageal connectives of *Aglaurides*, and that in the nerve cord such cells are rare, only two having, so far, been found.

II.—The Size of the Giant Cells of *Aglaurides*.

The giant cells of the first couple are usually small, 30 to 50 μ in diameter; those of the second couple are generally the largest cells in the specimen, their mean diameter varying in different worms from 70 to 93 μ; those of the third couple have

a diameter of 55 to 85 μ, of the fourth 35 to 60 μ, of the fifth 20 to 40 μ, while the cells in the sixth segment of the largest specimen and the secondary cells in the first and second segments of the same specimen are 20 to 27 μ in diameter.

The giant cells of *Aglaurides* apparently enlarge rapidly soon after they are differentiated, for some of them are large even in young worms—the largest giant cells of a specimen 35 mm. long have a mean diameter of 80 μ—but they subsequently grow only slowly and for a comparatively short time; they have, in fact, already almost attained their maximum size (their mean diameter being about 90 μ) when the worm is about 14 cm. long. Henceforward the size of the giant cells remains practically unchanged: the two largest cells of a specimen 315 mm. in length are 90 and 93 μ in diameter respectively. In *Halla*, as already stated (p. 452), there is a progressive increase in the size of the giant cells, which is roughly proportional to the growth in length of the worm, at any rate up to a certain point; but, as the above figures show, there is no such increase in the size of the giant cells of *Aglaurides* after the specimen has passed a length of 35 mm. (*cf.* figs. J, K, L, M). Although the giant cells of young specimens, say, up to 45 mm. in length, of *Aglaurides* are distinctly larger than those of examples of *Halla* of the same length, this difference is more than neutralised before the worms reach a length of 140 mm. Henceforward there is little or no change in the size of the giant cells of *Aglaurides*, but those of *Halla* continue to increase in size for a considerable period, until the specimen is 30 to 40 cm. in length, by which time they have attained a diameter of 135 μ. There is thus a great difference in size between the largest giant cells of adult examples of *Aglaurides* and *Halla*.

III.—The General Morphology of the Giant Cells of *Aglaurides*. (Plate 35, fig. 32.)

The giant cells of *Aglaurides* are generally pear-shaped, though they may be somewhat flattened in one plane. Each has a single process—the giant fibre. These cells agree with those of *Halla* in the main features, but differ in some points of detail of their structure.

The nucleus is large and vesicular, measuring from 20 to 30 μ in diameter; its membrane is thin but complete, and its chromatin is moderate or somewhat scanty in amount. There is generally one nucleolus 4 to 5 μ in diameter, but in three or four cases, in addition to the principal nucleolus, one or two small nucleoli are also present (fig. 30). The division of the nucleolus into two substances (*cf.* p. 470), differentiated by their staining reactions, is either very faintly marked or absent altogether.

In the majority of the giant cells of *Aglaurides* there is a clearly defined perinuclear zone of protoplasm which stains more deeply than the rest of the protoplasm, and at the outer margin of which the perinuclear neurofibrillar network is well seen. This zone agrees so closely with that of the giant cells of *Halla* (p. 467), except that it does

not exhibit quite so many vacuoles, that no detailed description of it is necessary. This perinuclear zone in most cells passes abruptly into the general cytoplasm, but in others the chromophilous substance is heaped up in a uniformly dense mass in contact with, and all round, the nuclear membrane, and gradually shades off into the general cytoplasm. In a few of the smaller cells the perinuclear zone is feebly differentiated from the rest of the cytoplasm.

The general protoplasm of the giant cells of *Aglaurides* is less granular than that of the giant cells of *Halla*, and in only one cell of the former was a patch of larger deeply staining granules found (*cf.* p. 465). As I have not had the opportunity of seeing the giant cells of *Aglaurides* in the living or fresh condition, I am unable to state whether they contain any yellow pigment granules in life.

Numerous vacuoles are present throughout the general cytoplasm, and occasionally structures, which seem to be homologous with HOLMGREN'S "Saftkanälchen," may be seen at the periphery of the cell and passing to a greater or less distance into its cytoplasm (figs. 31, 33). In tangential sections, in which these canals can be well seen in the peripheral portion of the cell, these structures are obviously not ordinary vacuoles, but elongate channels, sometimes extending for considerable distances. They have sharply defined margins, as seen in section, but I have never observed the presence of any special lining film or membrane.

A specially differentiated peripheral zone of protoplasm, such as is present in the giant cells of *Halla* (p. 466), is rarely exhibited by those of *Aglaurides*, and when it is present it is very feebly marked (*cf.* figs. 18, 32).

In two of the giant cells of *Aglaurides* there is a more darkly stained area of protoplasm, almost in the form of a cone, whose base is applied to or lies near the nucleus, and whose apex extends almost into the axone (fig. 32). In all the other cells examined the cone of origin of the axone does not present any features which distinguish it from the general cytoplasm.

IV.—The Giant Fibres of *Aglaurides*.

The giant fibres are similar in their course and size to those of *Halla*, at any rate in the first fifteen or twenty segments of the worm. Some of the fibres from the smaller giant cells may disappear comparatively soon, for instance before reaching the twentieth segment, and only the larger giant fibres run along the rest of the nerve cord.

The branches of the giant fibres are more slender than those of *Halla*, and their course and contents even more difficult to investigate (see p. 473). As their investigation did not promise to materially further the main results of this work, I have spent comparatively little time upon it. As far as my examination of them goes, their course agrees with that of the corresponding structures in *Halla*. Besides these slender branches, single neurofibrillæ may occasionally be seen issuing from the giant fibre into the neuropile, where they are soon lost to view (fig. 35).

V.—The Sheath of the Giant Cells and of the Giant Fibres of *Aglaurides*.

The sheath of the giant cell of *Aglaurides* has a looser texture than that of the giant cells of *Halla*, and in some specimens is divisible into two portions, an outer one with thicker fibrillæ, and an inner composed of thinner, more faintly stained fibrils (fig. 30). The principal components of the sheath are glia fibrils, among which are large glia nuclei, but there is little of the granular glia protoplasm remaining, except in the immediate neighbourhood of the glia nuclei.

The sheath of the giant fibres of *Aglaurides* (fig. 34) contains the same elements as have been already described in that of the giant fibres of *Halla* (p. 474).

VI.—The Neurofibrillæ of the Giant Cells and Giant Fibres of *Aglaurides*.

In the majority of the giant cells of *Aglaurides* the intracellular fibrillar network is divisible into two more or less clearly differentiated portions—the perinuclear network situated at the outer margin of the perinuclear zone and the general network found throughout the rest of the protoplasm, with the exception of a narrow peripheral zone. The perinuclear network is close meshed and its strands are more slender than those of the corresponding network in the giant cells of *Halla*. The general network is rather more open meshed than the perinuclear one and its strands are thinner, in some cells they are of great tenuity. In two or three cells the general network presents what may be termed a zonary differentiation, a zone of the network situated about half-way between the nucleus and the periphery of the cell being composed of somewhat thicker fibrils (fig. 31). In two cells there are slightly stronger fibrils a short distance within and parallel to the periphery of the cell. A diffuse network, not divisible into specialised regions (perinuclear and general) is exhibited only by a very few giant cells and these are generally small ones.

The preparations of the giant cells of *Aglaurides* have been carefully studied with regard to the fibrillar elements which perforate the sheath and enter the cytoplasm. Many of these fibrils are darkly and sharply stained long filaments which can be seen running more or less radially through a portion of the sheath before they enter the protoplasm of the giant cell, that is, they are not processes simply given off from the inner face of the sheath into the cell, but may be seen in the middle or outer portion of the sheath and traced almost directly radially through a portion of its substance before becoming free and entering the cytoplasm of the giant cell (fig. 30). When they have penetrated a greater or less distance into the cell they divide or give off branches which apparently become continuous with the general or even the perinuclear intracellular network. There are several cases which do not seem to admit of any other interpretation and which consequently point to the nervous nature of these particular fibrillar ingrowths (but see the remarks on this subject on pp. 480–483).

The numerous short radial glia fibrils which are present in the peripheral zone of the giant cells of *Halla* are practically absent in those of *Aglaurides*. Somewhat larger and clustered fibrils, probably glial, are present in some of the cells of the latter, associated with one or more sinuses or canals (fig. 31).

The majority of the fibrillar ingrowths into the giant cells of *Aglaurides* are of the nature of the long clearly stained filaments above described, and in most of the cells in which these entrant fibrils are clearly seen there are several of them at the sides of the cone of origin of the axone. These fibrils, whose staining reactions are very similar to those of the axone, are clearly traceable into association with the intracellular network. In several cases these fibrils may be traced backwards from the cell a considerable distance towards and nearly to the neuropile. One or two preparations in particular suggest that these and other entrant fibrils come from the direction of the neuropile and run chiefly in the sheath of the axone, into the pericellular sheath, where they spread and subsequently enter the protoplasm of the giant cell at various points, but, as already remarked (see p. 483), the methods at present available are inadequate to enable one to definitely determine the nature and place of origin of these fibrils.

The giant cells of *Aglaurides* clearly show that each of the fibrillæ of the axone is formed as the result of the fusion of a number of very slender primitive fibrillæ. These latter usually arise from the general network, but in a few cases there seems to be a somewhat more intimate association of a number of the axone fibrillæ with the perinuclear network, but even in these cases the majority of the axone fibrillæ arise from the strands issuing from the general network.

The fibrils which issue from the cell by way of the axone (figs. 57, 58) are stouter than those of the intracellular network and are generally of approximately equal thickness, but in a few cases there are from one to four fibrils slightly thicker than the rest. It has not been possible in all cases to trace these backwards to their origin in the giant cells, but in the case of those which could be so followed the thicker fibrils were generally found to be associated in their origin with some stouter strand or strands of the network or were formed by the union of a greater number of primitive fibrils than appear to take part in the formation of the neighbouring somewhat thinner fibrillæ in the same axone. The giant fibres in which these thicker fibrillæ occur are not distinguished in any other way from those in which the fibrils are of equal thickness.

The number of fibrillæ in the different giant fibres varies according to the size of the cell from which the fibre originates. The small giant fibres from the smaller cells contain few fibrils, not more than about ten, but in the larger ones I have counted sixteen, eighteen, and twenty, and in one case there appear to be more, about thirty, but owing to their close proximity it was not possible to count them.

The neurofibrillæ of the giant fibres of *Aglaurides* are slightly thicker than those of *Halla*. In three or four cases in which their thickness was carefully estimated

they are about 0·3 μ in breadth shortly after leaving the giant cell, but they are rather thinner in the more distal portions of the giant fibre.

In a large number of the longitudinal sections of *Aglaurides* the neurofibrillæ are extremely clearly seen, forming a sinuous bundle in each giant fibre (figs. 34, 35, 59). The fibrillæ occupy only the central part of the giant fibre, the bundle being about 4 to 8 μ in diameter and the lumen of the fibre 35 to 40 μ in the case of the large giant fibres (fig. 36). The interfibrillar substance, here clearly seen, is a faintly stained and almost homogeneous matrix in which the fibrillæ lie. At the sides of the fibrillar bundle, between it and the wall of the giant fibre, are the remains of the perifibrillar substance, forming a thin-stranded, slightly granular meshwork, an artefact produced by coagulation of the substance on treatment with fixing reagents. The interfibrillar and perifibrillar substance are portions of the protoplasm of the axis cylinder of the giant fibre (p. 487), which it is convenient to name by APÁTHY'S terms. The latter substance, judging by its condition in the sections, must be in some cases very fluid, almost watery, in life, as it contains only a small quantity ot coagulable material.

The neurofibrillar contents of the branches of the giant fibres have not been investigated for reasons already given (p. 495).

VII.—The Posterior Giant Cells and Giant Fibres of *Aglaurides*.

In addition to the anterior giant cells described above (p. 491), there are, in *Aglaurides*, about six to eight posterior giant cells, the mean diameter of which is 25 to 30 μ, and whose position and relations are similar to those of *Halla* (p. 488). The axones, on issuing from their respective cells, cross to the opposite side of the cord and run forwards in the lateral regions of the neuropile. An account of the arrangement, histology, and fibrillar contents of these cells and fibres will be given in a future portion of this work.

5. SUMMARY.

1. Two series of giant cells are present in *Halla parthenopeia*, an anterior series in the first few ganglia and a posterior series, consisting of about six to eight small giant cells, in the last few segments (p. 488). The anterior series only is referred to in the following statement.

2. The giant cells, recognisable by reason of their large size and thick enveloping sheath, arise in segmental couples, one couple in each of the anterior ganglia of the nerve cord. In specimens 14 and 44 mm. long, five such couples are already present, and as the worm increases in length other couples are formed in the succeeding segments until a maximum of eight couples is attained. These, which may be called the primary giant cells, have a definite situation in the posterior portion of the segment to which they belong: each couple is so placed that the posterior cell lies close to the hinder border of the segment. While the last three couples of giant cells

are being formed there are from two to four smaller secondary giant cells differentiated at the anterior end of the nerve cord close to the point of entry of the œsophageal connectives and, in a considerable number of specimens, a small secondary cell appears in the anterior or middle portion of one or more ganglia already possessing a couple of primary giant cells. The number of giant cells in a full-grown specimen is usually fifteen to eighteen, but specimens with twenty and twenty-one cells are recorded (pp. 447–451).

3. There is a progressive increase in the size of the primary giant cells until the worm has attained a length of 30 to 40 cm., by which time the giant cells seem to have reached their maximum size, for they are as large in worms of this length as in others whose length is two or three times as great. The giant cells vary greatly in size in the same adult individual. The secondary cells are small (30–55 μ in diameter), the primary giant cells of the first couple are about 80 μ in diameter, those of the second and third couples are usually the largest (130–150 μ), while those of the fourth are almost equally well developed; the cells of the succeeding couples exhibit a progressive diminution in size (pp. 452, 453).

4. The chlorogogen cells around the nerve cord of *Halla* contain: (1) refringent fat droplets in which olein largely predominates; (2) granules which are composed of a resistant substance impregnated with a yellow pigment (which is not an ordinary lipochrome), both of which are of unknown nature. Fat is generally associated, possibly only as an enveloping film, with the granules, some of the larger of which are composite, being formed of two or three smaller granules closely apposed.

Clusters of similar bodies, some colourless and others yellow, the latter largely predominating, are found in the cellular parts of the nerve cord. The colourless ones probably consist largely of olein, the yellow ones correspond in their reactions to the similar granules in the chlorogogen cells. The small cells in which these bodies of both kinds occur are so fully occupied by the bodies that only a trace of the protoplasm can be seen along with the nucleus of the cell. These cells are apparently small specialised cells of a type similar to the chlorogogen cells.

Smaller yellow granules, which react in a similar way to those above mentioned, are found in the giant cells aggregated into one or two excentrically placed masses or forming an indefinite zone around the whole or a portion of the nucleus. Similar minute yellow granules are present in many of the ganglion cells, usually at the end of the cell opposite the axone. A small amount of fat is usually associated with these yellow granules which, consequently, stain red with Sudan or Scharlach; but fat is possibly not an essential constituent of the yellow granules; under certain conditions it appears to be almost wanting, and, when present, may form merely an enveloping film.

The substance and pigment of the yellow granules present in the giant and ganglion cells are closely similar to, if not identical with, those of the chlorogogen granules, a fact which indicates that the granules in the nerve cells are probably insoluble products of metabolism (pp. 454–462).

5. Small rounded granules, which stain blue with methylene or toluidin blue, are present in the cytoplasm of the giant cells, except in a peripheral zone of varying width in different cells, in which they are absent or very sparse. These chromophilous bodies, which are not aggregated into clusters or flakes as in many vertebrate nerve cells, are present in varying amount in the cells of different specimens, and even in the several cells of the same specimen, a variation no doubt dependent upon the functional condition of the cell just previous to the time of fixation. The granules are found in greatly increased mass in a specialised perinuclear zone, which is distinguishable even in the living cell by reason of its greater refringency. This zone is usually about 3 to 5 μ in width, concentric with the nucleus, and its outer margin almost entire; but the zone may be larger and ovoid in shape, with the nucleus in its broader end. There is probably a diffusion or extrusion from the nucleus of some substance, which is rapidly converted into the basophile granules present in such great numbers in this specialised zone of protoplasm. Closely associated with and practically bounding the outer edge of this zone is the perinuclear network of neurofibrillæ, which is thus in a position in which rapid and extensive nutrition of this, which is the most obvious, portion of the intracellular neurofibrillar network, will be facilitated. Vacuoles are generally present in the general and perinuclear protoplasm, but are seldom seen in the peripheral zone, in which, however, structures resembling sinuses are occasionally met with. The nucleus of the giant cell is large and vesicular; the nucleolus is, in many cases, divisible into two portions differentiated by their staining reactions, acidophile bodies being enveloped by a basophile substance, forming a "double nucleolus" (pp. 465–470).

6. Each giant fibre, after leaving the giant cell from which it arises, crosses the cord to the opposite side and then turns gradually towards the middle line of the cord and runs posteriorly. One or more small branches, which pass into the neuropile, are frequently present at the angle of decussation. The giant fibres run for a considerable distance (which varies in different specimens) in two groups: one group, consisting usually of about eight to ten fibres, lies in the dorsal region of the cord, the other, consisting of about six fibres, in the middle or ventral portion of the fibrous part of the cord. Giant fibres run practically the whole length of the nerve cord: some of the largest ones, from two to six in different specimens, may reach to within 1 or 2 mm. of the posterior end of the worm, the others having successively tapered and disappeared after running various distances down the cord. The giant fibres generally taper and disappear among the ordinary nerve fibres, but those which terminate in the last 1 or 2 mm. of the worm may end rather abruptly. The branches, which issue ventrally from the giant fibres situated in the dorsal region of the cord, fork and one twig passes to the right and the other to the left; the twigs taper, their sheath disappears, and the protoplasmic axis of the twig, which is by this time only about 1 μ in diameter, is lost to view in the lateral or ventro-lateral regions of the fibrous part of the cord; in no case could it be traced into a spinal nerve (pp. 471–473).

7. The sheath of the giant cell and giant fibre is not blackened by the action of osmic acid. It consists of glia fibrils: those in the outer portion of the sheath are usually stout and elongate, in the cell-sheath they are more or less concentric and interlaced, but in the fibre sheath they are chiefly longitudinal; in the inner portion of the sheath of the cell and fibre there are thinner, closely interlaced, glia fibrils and here the granular remains of the glia protoplasm are usually more clearly seen. Glia nuclei are found in both inner and outer portions of the sheath (pp. 474, 475)..

8. The neurofibrillar network in the giant cells is divisible into two portions: (1) a central portion, usually situated in close proximity to the nucleus; and (2) a more extensive, wider meshed and generally more slender stranded meshwork in the general cytoplasm. The perinuclear network is found at the margin of the perinuclear zone and consists of moderately stout fibrillæ, about 0·2 to 0·3 μ in thickness, which form a close network from which no fibrils pass towards the nucleus, but from which numerous fibrils pass outwards and merge into the general network. The fibres of the general network are usually about one-half the thickness of those of the perinuclear network (pp. 477–480).

Sharply and deeply stained fibrillæ penetrate the sheath of the giant cell, enter the cell and are traceable a long way (40 μ) into the cytoplasm, even as far as the perinuclear network. These fibrils seem to be continuous with the intracellular network and are therefore apparently neurofibrillæ (see pp. 481, 482). Numerous other fibrils also enter the cell from the inner border of the sheath; these are very short (about 5 μ in length) and slender and their branches form a meshwork in the peripheral zone of the protoplasm, to which zone they are generally restricted; their structure and relations suggest that they are glia fibrils. Somewhat longer fibrils of a nature similar to the latter are occasionally seen entering a giant cell accompanied by one or more sinuses (pp. 480–483).

From the fibrillar network in the cell neurofibrillæ pass into the cone of origin of the axone; they are at first very slender (primitive fibrillæ), but as they pass towards the axone stouter fibrils are formed, each due to the fusion of several slender ones, and may be traced into and along the giant fibre. The number of fibrillæ issuing from the cell varies with the size of the cell and consequently is, to some extent, related also to that of the giant fibre in which they lie (for the smaller cells give rise to smaller fibres, but the diameter of any giant fibre is by no means constant throughout its course). Only a few fibrillæ, from six to ten, may be counted in the giant fibres from the smaller anterior cells; these form a bundle 2 to 3 μ in diameter, lying in a giant fibre the internal diameter of which is 7 to 10 μ. In the larger fibres a considerably greater number of neurofibrillæ is present, namely, from a dozen to twenty or occasionally nearly thirty. Judging from the preparations (for the neurofibrillæ have never been seen in the living or fresh giant fibres), the bundle of neurofibrillæ does not fill the lumen of the giant fibre in life, but occupies, in different cases, from one-fourth to three-fourths of the internal diameter of the fibre, the

remaining space being filled with the semi-fluid, finely granular, perifibrillar substance. Between the fibrillæ there is a more homogeneous interfibrillar substance. The fibrillæ in a giant fibre are usually all of the same thickness, but in several fibres one to three fibrils thicker than the rest are seen ; these usually originate from thicker or more numerous strands from the intracellular network. The neurofibrillæ of the giant fibres of *Halla* are usually from 0·2 to 0·25 μ thick as they leave the giant cell to enter the giant fibre, but they are distinctly thinner in the more distal portions of the fibre. In worms killed fully extended the neurofibrillæ are practically straight, but in most specimens they have a sinuous course.

The contents of the giant fibre are the equivalent of the axis cylinder of an ordinary nerve fibre, being a direct continuation of the protoplasm of the giant cell; moreover, the structure of the axis cylinder of the giant fibre is very similar to that of the axis cylinder of a medullated nerve fibre, except that in the former there is nothing comparable to the Ranvier's nodes of the latter.

The branches of the giant fibres apparently end in the neuropile (see above, p. 500), their sheath disappears, and their fibrillæ probably enter the neuropile (in a similar manner to those of branches of the "sensorische Schläuche" of *Hirudo* described by Apáthy and Bethe), but this could not be definitely established (pp. 485–488).

9. There are in *Aglaurides fulgida*, as in *Halla*, anterior and posterior giant cells (the latter are six to eight in number, the former only are referred to in the following statement).

The giant cells of *Aglaurides* are segmentally arranged: in the youngest specimens examined, 35 and 45 mm. long, there are already four segmental couples of well-developed giant cells ; other specimens, 50 to 200 mm. long, have five couples, and in the largest specimen examined, which is 315 mm. in length, there are primary giant cells in each of the first six segments, and two secondary cells are also present in this specimen, but the latter are present only in this one out of eight specimens examined. The giant cells of *Aglaurides* seem to have attained their maximum size (about 90 μ in diameter) when the worm has reached a length of 14 cm. (pp. 491–494). These giant cells agree with those of *Halla* in the main features, but differ in some points of detail, of their structure. The general protoplasm is less granular, and a definite peripheral zone of protoplasm is rarely differentiated. In addition to the large nucleolus, one or more smaller ones may also be present (pp. 494, 495).

The perinuclear network is close meshed, and its strands are more slender than those of the corresponding network in the giant cells of *Halla*. The general network is rather more open meshed than the perinuclear one, and its strands are thinner ; in some cells they are of great tenuity. Fibrils may be seen in the outer or middle portion of the sheath of some of the giant cells, and traced almost radially through a portion of the sheath and into the cytoplasm of the cell, where they divide and apparently become continuous with the general, or even with the perinuclear, network. The fibrils which fuse to form the axone fibrillæ arise from the general

and perinuclear networks. The fibrillæ in the axone are generally all of the same thickness, but in several of the giant fibres there are from one to four fibrils thicker than the rest. The small giant fibres from the small giant cells contain few fibrils (not more than 10), but in the larger ones 16, 18, and 20 were counted, and in another case there were approximately 30. The neurofibrillæ are about 0·3 μ thick shortly after leaving the giant cell, but they are thinner in the more distal parts of the giant fibre, where they form a sinuous bundle occupying the central portion of the giant fibre, the bundle being 4 to 8 μ in diameter in a large giant fibre 35 to 40 μ in diameter. Single neurofibrillæ are given off from the bundle, penetrate the sheath of the giant fibre, and issue into the neuropile (pp. 496–498).

The morphology and histology of the posterior giant cells and fibres of *Halla* and *Aglaurides*, and of certain other elements of the nervous system of these worms, will be described in a future portion of this work.

In conclusion, I beg to express my grateful acknowledgments to Herr Geheimrath Professor Dohrn for placing at my disposal a table in the Zoological Station in Naples in 1900; to the British Association for the use of the "British Association Table" in the Naples Station in 1906; to the Government Grant Committee of the Royal Society, the Earl of Moray Endowment Fund of the University of Edinburgh, and the Carnegie Trust for the Universities of Scotland, for grants in aid of the work, and to the two last named for liberal help towards the cost of reproduction of the figures illustrating this memoir.

6. LIST OF REFERENCES TO THE LITERATURE.

Abel, M. "Beiträge zur Kenntnis der Regenerationsvorgänge bei den limicolen Oligochäten," in 'Zeits. Wiss. Zool.,' Bd. 73, pp. 1–74, Taf. 1–3, 1903.

Allen, E. J. "Studies on the Nervous System of Crustacea. I. Some Nerve-elements of the Embryonic Lobster," in 'Quart. Journ. Micr. Sc.,' vol. 36, pp. 461–482, Plates 35, 36, 1894.

Idem. "The Anatomy of *Pœcilochætus*, Claparède," *ibid.*, vol. 48, pp. 79–151, Plates 7–12, 1904.

Andrews, E. A. "Some Abnormal Annelids," *ibid.*, vol. 36, pp. 435–460, Plates 32–34, 1894.

Apáthy, S. "Das leitende Element des Nervensystems und seine topographischen Beziehungen zu den Zellen," in 'Mitt. Zool. Stat. Neapel,' Bd. 12, pp. 495–748, Taf. 23–32, 1897.

Ashworth, J. H. "The Anatomy of *Arenicola assimilis* . . . .," in 'Quart. Journ. Micr. Sc.,' vol. 46, pp. 737–785, Plates 36, 37, 1903.

Idem. "Arenicola," Liverpool Marine Biological Committee, Memoir XI, London, 1904.

Idem. "The Giant Nerve Cells and Fibres of *Halla parthenopeia*," in 'Proc. Roy. Soc. London,' B, vol. 80, pp. 463, 464, 1908. [Abstract of present memoir.]

ATTEMS, C. G. "Beiträge zur Anatomie und Histologie von *Scololepis fuliginosa*, Clap.," in 'Arb. Zool. Inst. Wien,' T. 14, pp. 173–210, 4 Taf., 1903.

BEDDARD, F. E. "A Monograph of the Order of Oligochæta," Oxford, 1895.

BENHAM, W. B. "Studies on Earthworms," in 'Quart. Journ. Micr. Sc.,' vol. 26, p. 293, 1886.

BETHE, A. "Das Molybdänverfahren zur Darstellung der Neurofibrillen und Golginetze im Centralnervensystem," in 'Zeits. Wiss. Mikros.,' Bd. 17, pp. 13–35, 1900.

Idem. "Allgemeine Anatomie und Physiologie des Nervensystems," Leipzig, 1903.

BOCHENEK, M. A. "Untersuchungen über das Zentral-Nervensystem der Wirbellosen," in 'Bull. Acad. Sc. Cracovie, Classe Math. Natur.,' pp. 205–220, 2 figs., Plate 5, 1905.

BRUNOTTE, C. "Recherches anatomiques sur une espèce du genre *Branchiomma*," in 'Travaux Stat. Zool. Cette,' 77 pages, 2 plates, 1888.

BUCHHOLZ, R. "Bemerkungen über den histologischen Bau des Centralnervensystems der süsswasser Mollusken," in 'Arch. Anat. Physiol.,' Jahrg. 1863, pp. 234–309, Taf. 6–8, 1863.

BÜLOW, C. "Die Keimschichten des wachsenden Schwanzendes von *Lumbriculus variegatus*," in 'Zeits. Wiss. Zool.,' Bd. 39, pp. 64–96, Taf. 5, 1883.

CAJAL, S. RAMÓN. "Neuroglia y neurofibrillas del *Lumbricus*," in 'Trabajos Labor. Investig. Biol. Univ. Madrid,' T. 3, pp. 277–285, 4 figs., 1904.

Idem. "Variaciones morfológicas del reticulo nervioso de invertebrados y vertebrados sometidos á la accion de condiciones naturales," *ibid.*, T. 3, pp. 287–297, 5 figs., 1904A.

Idem. "Las celulas del gran simpatico del hombre adulto," *ibid.*, T. 4, pp. 79–104, 1905.

Idem. "Studien über die Hirnrinde des Menschen," 5 Heft, Leipzig, 1906.

CAMERON, J "The Development of the Vertebrate Nerve-cell: a Cytological Study of the Neuroblast-nucleus," in 'Brain,' vol. 29, pp. 332–362, 4 plates, 1906.

CERFONTAINE, P. "Contribution à l'étude du système nerveux central du Lombric terrestre," in 'Bull. Acad. Roy. Belgique,' T. 23, pp. 742–752, Plates 1, 2, 1892.

CLAPARÈDE, E. "Études anatomiques sur les Annélides, Turbellariés, etc., observés dans les Hébrides," in 'Mem. Soc. Phys. Hist. Nat. Genève,' T. 16, pp. 71–164, Plates 1–7, 1861.

Idem. "Recherches anatomiques sur les Oligochètes," *ibid.*, T. 16, pp. 217–291, Plates 1–4, 1862.

Idem. "Beobachtungen über Anatomie und Entwickelungsgeschichte wirbelloser Thiere an der Küste von Normandie angestellt," Leipzig, 1863.

Idem. "Les Annélides chétopodes du Golfe de Naples," Genève et Bale, 1868.

CLAPARÈDE, E. "Histologische Untersuchungen über den Regenwurm (*Lumbricus terrestris*, Linné)," in 'Zeits. Wiss. Zool.,' Bd. 19, pp. 563–624, Taf. 43–48, 1869.

Idem. "Les Annélides chétopodes du Golfe de Naples," Supplement, Genève, 1871.

Idem. "Recherches sur la structure des Annélides sédentaires," in 'Mem. Soc. Phys. Hist. Nat. Genève,' T. 22, pp. 1–200, 15 plates, 1873.

COLLIN, A. "*Criodrilus lacuum*, Hoffm.," in 'Zeits. Wiss. Zool.,' Bd. 46, pp. 471–497, Taf. 33, 1888.

CUNNINGHAM, J. T. "The Significance of Kupffer's Vesicle, with Remarks on other Questions of Vertebrate Morphology," in 'Quart. Journ. Micr. Sc.,' vol. 25, pp. 1–14, Plate 1, 1885.

Idem. "On some Points in the Anatomy of Polychæta," *ibid.*, vol. 28, pp. 239–278, Plates 17–19, 1888.

EHLERS, E. "Die Borstenwürmer," Leipzig, 1864–1868.

EHRENBERG, C. G. "Beobachtung einer bisher unbekannten auffallenden Structur des Seelenorgans bei Menschen und Thieren," in 'Abh. Phys.-math. Kl. Königl. Akad. Wiss. Berlin,' aus dem Jahre 1834, Berlin, 1836.

EHRLICH, P., etc. 'Encyklopädie der mikroskopischen Technik.' Berlin und Wien, 1903.

EISIG, H. "Capitelliden," in 'Fauna u. Flora, Golf Neapel,' Monogr. 16, Berlin, 1887.

Idem. "*Ichthyotomus sanguinarius*, eine auf Aalen schmarotzende Annelide," *ibid.*, Monogr. 28, Berlin, 1906.

EMBLETON, ALICE L. "On the Structure and Affinities of *Echiurus unicinctus*," in 'Trans. Linn. Soc. Lond. Zool.,' vol. 8, pp. 77–97, Plates 7–10, 1900.

EMERY, C. "La régénération des segments postérieurs du corps chez quelques Annélides polychètes," in 'Arch. Ital. Biologie,' T. 7, pp. 395–403, 1886.

FAUVEL, P. "Recherches sur les Ampharétiens," in 'Bull. Sc. France et Belgique,' T. 30, pp. 277–489, Plates 15–25, 1897.

Idem. "Sur le pigment des Arénicoles," in 'C. R. Acad. Sc. Paris,' T. 129, pp. 1273–1275, 1899.

FRIEDLÄNDER, B. "Beiträge zur Kenntniss des Centralnervensystems von *Lumbricus*," in 'Zeits. Wiss. Zool.,' Bd. 47, pp. 47–84, Taf. 9, 10, 1888.

Idem. "Über die markhaltigen Nervenfasern und Neurochorde der Crustaceen und Anneliden," in 'Mitt. Zool. Stat. Neapel,' Bd. 9, pp. 205–265, Taf. 8, 1891.

Idem. "Beiträge zur Physiologie des Centralnervensystems und des Bewegungsmechanismus der Regenwürmer," in 'Arch. Ges. Physiol.,' Bd. 58, pp. 168–206, 1894.

Idem. "Altes und Neues zur Histologie des Bauchstranges des Regenwurms," in 'Zeits. Wiss. Zool.,' Bd. 58, pp. 661–693, Taf. 40, 1894A.

FRIEDLÄNDER, B. "Über die Regeneration herausgeschnittener Theile des Centralnervensystems von Regenwürmern," *ibid.*, Bd. 60, pp. 249–283, Taf. 13, 14, 1895.

GAMBLE, F. W., and ASHWORTH, J. H. "The Anatomy and Classification of the Arenicolidæ, . . . ," in 'Quart. Journ. Micr. Sc.,' vol. 43, pp. 419–569, Plates 22–29, 1900.

GARMAN, H. "On the Anatomy and Histology of a New Earthworm, *Diplocardia communis*, g. and sp. n.," in 'Bull. Illinois Lab. Nat. Hist.,' vol. 3, pp. 47–77, Plates 1–5, 1888.

GRAF, A. "The Physiology of Excretion," in 'Biol. Lectures, Wood's Holl, 1896–7,' pp. 79–107, Boston, 1898.

Idem. "Hirudineen-Studien," in 'Nova Acta K. Leop. Car. D. Akad. Naturf. Halle,' Bd. 72, No. 2, 1899.

GREEF, R. "Über die borstentragenden Gephyreen.—Die Organisation der Echiuriden," in 'Sitzungsber. Ges. Bef. Ges. Naturw. Marburg,' No. 6, 1872.

Idem. "Die Echiuren," in 'Nova Acta K. Leop. Car. D. Akad. Naturf. Halle,' Bd. 41, Abth 2, pp. 1–172, Taf. 16–24, 1879.

HAECKEL, E. "Ueber die Gewebe des Flusskrebses," in 'Arch. Anat. Physiol.,' Jahrg. 1857, pp. 469–568, 1857.

HALLER, B. "Untersuchungen über marine Rhipidoglossen. II. Textur des Centralnervensystems und seiner Hüllen," in 'Morph. Jahrb.' Bd. 11, pp. 321–436, Taf. 17–24, 1886.

Idem. "Beiträge zur Kenntniss der Textur des Centralnervensystems höherer Würmer," in 'Arb. Zool. Inst. Wien,' T. 8, pp. 175–312, 5 Taf., 1889.

HALPERN, B. "Das Hüll- und Stützgewebe des Bauchmarks bei *Astacus fluviatilis*," *ibid.*, T. 14, pp. 423–442, 12 figs., 1903.

HAMAKER, J. I. "The Nervous System of *Nereis virens*, Sars.," in 'Bull. Mus. Comp. Zool. Harvard,' vol. 32, pp. 89–124, 5 plates, 1898.

HANNOVER, A. "Mikroskopiske Undersögelser af Nervesystemet." Kjöbenhavn, 1842.

HASWELL, W. A. "Observations on some Australian Polychæta," in 'Proc. Linn. Soc. N. S. Wales,' vol. 10, pp. 733–756, Plates 50–55, Sydney, 1886.

HATAI, S. "A Note on the Significance of the Form and Contents of the Nucleus in the Spinal Ganglion Cells of the Fœtal Rat," in 'Journ. Comp. Neur., vol. 14, pp. 27–48, Plates 3, 4, 1904.

HEIDENHAIN, M. "Plasma und Zelle," Lief. I, Jena, 1907.

HELMHOLTZ, H. VON. "De Fabrica Systematis Nervosi Evertebratorum." Dissert Berolini, 1842.

HOLMGREN, E. "Zur Kenntnis der Spinalganglienzellen von *Lophius piscatorius*, Linn.," in 'Anat. Hefte,' Bd. 12 (Heft 38), 1899.

HOLMGREN, E. "Studien in der feineren Anatomie der Nervenzellen," *ibid.*, Bd. 15 (Heft 47), 1900.

Idem. "Beiträge zur Morphologie der Zelle," *ibid.*, Bd. 18, pp. 267–325, Taf. 17–26, 1901.

Idem. "Ueber die Trophospongien centraler Nervenzellen," in 'Arch. Anat. Physiol.,' Anat. Abth., pp. 15–32, Taf. 4–6, 1904.

IZUKA, A. "On some Points in the Organisation of *Ceratocephale osawai*, Iz.," in 'Annot. Zool. Japon.,' vol. 5, pp. 239–252, Plate 12, 1905.

JACOBI, R. "Anatomisch-histologische Untersuchung der Polydoren der Kieler Bucht," Inaug.-Dissert., Kiel, 1883.

JOEST, E. "Transplantationsversuche an Lumbriciden," in 'Arch. Entw.-mech. Org.,' Bd. 5, pp. 419–569, Taf. 6, 7, 1897.

JOSEPH, H. "Untersuchungen über die Stützsubstanzen des Nervensystems," in 'Arb. Zool. Inst. Wien,' T. 13, pp. 335–400, 4 Taf., 1902.

JOURDAN, E. "Études histologiques sur deux espèces du genre *Eunice*," in 'Ann. Sc. Nat., Zool.,' T. 2, pp. 239–304, Plates 12–16, 1887.

KEFERSTEIN, W. "Untersuchungen über niedere Seethiere," in 'Zeits. Wiss. Zool.,' Bd. 12, pp. 1–147, Plates 1–11, 1862.

KORSCHELT, E. "Über Regenerations- und Transplantations-Versuche an Lumbriciden," in 'Verh. Deutsch. Zool. Ges.,' pp. 79–94, 18 figs., Leipzig, 1898.

KOWALEVSKY, A. "Embryologische Studien an Würmern und Arthropoden," in 'Mém. Acad. St. Pétersbourg,' Sér. 7, T. 16, No. 12, 1871.

KRAWANY, J. "Untersuchungen über das Zentralnervensystem des Regenwurms," in 'Arb. Zool. Inst. Wien,' T. 15, pp. 281–316, 5 Taf., 1905.

KULAGIN, N. "Zur Anatomie der in Russland vorkommenden Regenwürmer," in 'Zool. Anz.,' Bd. 13, pp. 404–406, 1890.

LANG, A. "Lehrbuch der vergleichenden Anatomie der wirbellosen Thiere," Jena, 1894.

LANGERHANS, P. "Die Wurmfauna von Madeira," in 'Zeits. Wiss. Zool.,' Bd. 34, pp. 87–143, Taf. 4–6, 1880.

LEGENDRE, R. "Sur la présence de granulations dans les cellules nerveuses d'*Helix aspersa* et leur cylindraxe," in 'C.R. Soc. Biol. Paris,' T. 58, pp. 494–496, 1905.

Idem. "Sur un nouveau détail de la structure des cellules nerveuses d'*Helix pomatia*," *ibid.*, T. 60, pp. 488–490, 1906.

VON LENHOSSÉK, M. "Ursprung, Verlauf und Endigung der sensibeln Nervenfasern bei *Lumbricus*," in 'Arch. Mikr. Anat.,' Bd. 39, pp. 102–136, Taf. 5, 1892.

LEWIS, M. "Studies on the Central and Peripheral Nervous Systems of two Polychæte Annelids," in 'Proc. Amer. Acad. Arts and Sc.,' vol. 33, pp. 225–268, 8 plates, 1898.

LEYDIG, F. "Lehrbuch der Histologie," Frankfurt a/M., 1857.

Idem. "Vom Bau des thierischen Körpers," Tübingen, 1864; also "Tafeln zu vergleichenden Anatomie," Tübingen, 1864.

Idem. "Über den *Phreoryctes menkeanus* . . . .," in 'Arch. Mikr. Anat.,' Bd. 1, pp. 249–294, 1865.

Idem. "Die riesigen Nervenröhen im Bauchmark der Ringelwürmer," in 'Zool. Anz.,' 9, Jahrg., pp. 591–597, 1886.

MCGILL, C. "The Behavior of the Nucleoli . . . .," in 'Zool. Jahrb.,' Abth. Anat., Bd. 23, pp. 207–230, Taf. 13–15, 1906.

MCINTOSH, W. C. "On the Arrangement and Relations of the Great Nerve Cords in the Marine Annelids," in 'Proc. Roy. Soc. Edin.,' vol. 9, pp. 372–381, 1878.

Idem. "Beiträge zur Anatomie von *Magelona*," in 'Zeits. Wiss. Zool.,' Bd. 31, pp. 401–472, Taf. 29–38, 1878A.

MCMUNN, C. A. "Contributions to Animal Chromatology," in 'Quart. Journ. Micr. Sc.,' vol. 30, pp. 51–96, Plate 6, 1889.

MANN, G. "Physiological Histology," Oxford, 1902.

MEYER, E. "Zur Anatomie und Histologie von *Polyophthalmus pictus*, Clap.," in 'Arch. Mikr. Anat.,' Bd. 21, pp. 769–823, Plates 21, 22, 1882.

Idem. "Studien über den Körperbau der Anneliden. IV," in 'Mitt. Zool. Stat. Neapel,' Bd. 8, pp. 462–662, Plates 23–25, 1888.

MICHAELSEN, W. "Untersuchungen über *Enchytræus möbii* und andere Enchytræiden." Inaug.-Dissert., Kiel, 1886.

MICHEL, A. "Recherches sur la régénération chez les Annélides," in 'Bull. Sc. France et Belgique,' T. 31, pp. 245–420, Plates 13–19, 1898.

Idem. "Sur les canaux neuraux et les fibres nerveuses des Annélides," in 'Miscell. Biol. dédiées au Prof. Giard,' pp. 478–488, Plate 26, Paris, 1899.

MONCKBERG, G., and BETHE, A. "Die Degeneration der markhaltigen Nervenfasern der Wirbelthiere . . . .," in 'Arch. Mikr. Anat.,' Bd. 54, pp. 135–183, Taf. 8, 9, 1899.

NANSEN, F. "Anatomie und Histologie des Nervensystems der Myzostomen," in 'Jenaisch. Zeits. Naturw.,' Bd. 21, pp. 267–321, Taf. 19, 1887.

Idem. "The Structure and Combination of the Histological Elements of the Central Nervous System," in 'Bergens Museums Aarsb. f. 1886,' pp. 27–215, 11 plates, 1887A.

NASSE, D. "Beiträge zur Anatomie der Tubificiden," Inaug.-Dissert., Bonn, 1882.

OWSJANNIKOW, P. "Über die Nervenelemente und das Nervensystem des Flusskrebses (*Astacus fluviatilis*)," in 'Mém. Acad. Imp. Sc. St. Pétersbourg,' Classe Phys.-Math., vol. 10, No. 2, pp. 1–29, 1 plate, 1900.

PERRIER, E. "Études sur l'organisation des Lombriciens terrestres," in 'Arch. Zool. Expér.,' T. 3, 1874.

Idem. *Idem., ibid.*, T. 9, 1881.

PERRIER, E. 'Traité de Zoologie,' Fasc. 4, Paris, 1897.

PRENTISS, C. W. "A Case of Incomplete Duplication of Parts and Apparent Regulation in *Nereis virens*, Sars.," in 'Amer. Natural.,' vol. 35, pp. 563–574, 6 figs., 1901.

Idem. "Über die Fibrillengitter in dem Neuropil von *Hirudo* und *Astacus* . . . .," in 'Arch. Mikr. Anat.,' Bd. 62, pp. 592–602, Taf. 26, 1903.

PRUVOT, G. "Recherches anatomiques et morphologiques sur le système nerveux des Annélides polychètes," in 'Arch. Zool. Expér.,' Sér. 2, T. 3, pp. 211–336, Plates 11–16, 1885.

RABES, O. "Transplantationsversuche an Lumbriciden . . . .," in 'Arch. Entwick.-mech. Org.,' Bd. 30, pp. 239–352, Taf. 3–11, 1902.

RANDOLPH, H. "The Regeneration of the Tail in *Lumbricus*," in 'Journ. Morphology,' vol. 7, pp. 317–344, Plates 19–22, 1892.

RATZEL, F. "Beiträge zur anatomischen und systematischen Kenntniss der Oligochæten," in 'Zeits. Wiss. Zool.,' Bd. 18, pp. 563–591, Taf. 42, 1868.

REICHERT, K. B. "Bericht über die Fortschritte der mikroskopischen Anatomie im Jahre 1843," in 'Arch. Anat. Physiol.,' Jahrg. 1844.

REMAK, R. "Ueber den Inhalt der Nervenprimitivröhren," *ibid.*, Jahrg. 1843.

Idem. "Neurologische Erläuterungen," *ibid.*, Jahrg. 1844.

RETZIUS, G. "Zur Kenntniss des Nervensystems der Crustaceen," in 'Biol. Unters.,' N.F., Bd. 1, pp. 1–50, Taf. 1–14, 1890.

Idem. "Zur Kenntniss des centralen Nervensystems der Würmer," *ibid.*, Bd. 2, 1891.

Idem. "Das Nervensystem der Lumbricinen," *ibid.*, Bd. 3, pp. 1–16, Taf. 1–6, 1892.

ROBERTSON, W. F. "Normal and Pathological Histology of the Nerve Cell. Critical Digest," in 'Brain,' vol. 22, pp. 203–327, 1899.

ROHDE, E. "Histologische Untersuchungen über das Nervensystem der Polychæten," in 'Zool. Beiträge,' Bd. 2, pp. 1–81, Taf. 1–7, 1887.

Idem. "Ganglienzelle, Axencylinder, Punktsubstanz und Neuroglia," in 'Arch. Mikr. Anat.,' Bd. 45, 1895.

Idem. "Ganglienzellkern und Neuroglia," *ibid.*, Bd. 47, pp. 121–135, Taf. 9, 1896.

Idem. "Untersuchung über den Bau der Zelle. I.—Kern und Kernkörper," in 'Zeits. Wiss. Zool.,' Bd. 73, pp. 497–682, Taf. 32–40, 1903.

ROSIN, H. "Ein Beitrag zur Lehre vom Bau der Ganglienzellen," in 'Deutsch. Medic. Wochenschr.,' Jahrg. 22, pp. 495–497, 1896.

DE SAINT-JOSEPH. "Les Annélides polychètes des Côtes de Dinard. 3^{me} Partie," in 'Ann. Sc. Nat., Zool.,' T. 17, pp. 1–395, 13 plates, 1894.

SCHACK, F. "Anatomisch-histologische Untersuchung von *Nephthys cæca*, Fabricius," Inaug.-Dissert., Kiel, 1886.

SCHAEPPI, T. "Das Chloragogen von *Ophelia radiata*," in 'Jenaisch. Zeits. Naturw.,' Bd. 28, 1894.

SCHNEIDER, K. C. "Lehrbuch der vergleichenden Histologie der Tiere," Jena, 1902.

SCHRÖDER, G. "Anatomisch-histologische Untersuchung von *Nereis diversicolor*, O. Fr. Müll.," Inaug.-Dissert., Rathenow, 1886.

SCHULTZE, H. "Die fibrilläre Structur der Nervenelemente bei Wirbellosen," in 'Arch. Mikr. Anat.,' Bd. 16, pp. 57–111, Taf. 5, 6, 1879.

SCOTT, F. H. "The Structure, Microchemistry, and Development of Nerve Cells," in 'Trans. Canadian Inst.,' vol. 6, pp. 405–438, 1899.

SEITZ, P. "Der Bau von *Echiurus chilensis* (*Urechis* n.g., *chilensis*)," in 'Zool. Jahrb.,' Anat. Abth., Bd. 24, pp. 323–356, Taf. 29–31, 1907.

SEMPER, C. "Die Stammesverwandtschaft der Wirbelthiere und Wirbellosen," in 'Arb. Zool.-Zoot. Inst. Würzburg,' Bd. 2, pp. 25-76, Taf. 3–5, 1874.

Idem. "Die Verwandtschaftsbeziehungen der gegliederten Thiere. III . . . ," *ibid.*, Bd. 3, pp. 115–404, Taf. 5–15, 1876.

SIMON, C. "Recherches sur la cellule des ganglions sympathétiques des Hirudinées," in 'Internat. Monatschr. Anat. Physiol.,' Bd. 13, pp. 278–310, 1896.

SMALLWOOD, W. M. "Preliminary Report on the Cytology of Molluscan Nerve Cells," in 'Journ. Compar. Neurol.,' vol. 16, pp. 183–188, 1906.

SMALLWOOD, W. M., and ROGERS, C. G. "Studies on Nerve Cells. I.—The Molluscan Nerve Cell . . . ," *ibid.*, vol. 18, pp. 45–86, Plate 1, 1908.

SMITH, J. LORRAIN. "The Staining of Fat with Basic Aniline Dyes," in 'Journ. Path. Bacter.,' vol. 11, pp. 415–420, 1907.

Idem. "On the Simultaneous Staining of Neutral Fat and Fatty Acid by Oxazine Dyes," *ibid.*, vol. 12, pp. 1–4, 1907A.

SPENCER, W. B. "The Anatomy of *Megascolides australis*," in 'Trans. Roy. Soc. Victoria,' vol. 1, pp. 3–60, Plates 1–6, 1888.

SPENGEL, J. W. "Beiträge zur Kenntnis der Gephyreen," in 'Zeits. Wiss. Zool.,' Bd. 34, pp. 460–538, Taf. 23–26, 1880.

Idem. "*Oligognathus bonelliæ*, eine schmarotzende Eunicee," in 'Mitt. Zool. Stat. Neapel,' Bd. 3, pp. 15–52, Taf. 2–4, 1881.

STEEN, J. "Anatomisch-histologische Untersuchung von *Terebellides strœmii*, M. Sars," in 'Jenaisch. Zeits. Naturw.,' Bd. 16, pp. 201–246, Taf. 11–13, 1883.

TREADWELL, A. L. "Preliminary Note on the Anatomy and Histology of *Serpula dianthus* (Verrill)," in 'Zool. Anz.,' Bd. 14, pp. 276–280, 1891.

VEJDOVSKY, F. "Anatomische Studien an *Rhynchelmis limosella*, Hoffm.," in 'Zeits. Wiss. Zool.,' Bd. 27, pp. 332–361, Taf. 21–24, 1876.

Idem. "Beiträge zur Kenntniss der Tomopteriden," *ibid.*, Bd. 31, pp. 81–100, Taf. 6, 7, 1878.

Idem. "Beiträge zur vergleichenden Morphologie der Anneliden. I.—Monographie der Enchytraeiden," Prag, 1879.

VEJDOVSKY, E. "Untersuchungen über die Anatomie, Physiologie und Entwickelung von *Sternaspis*," in 'Denkschr. Akad. Wiss. Wien,' Math.-Naturw. Classe, Bd. 43, Abth. 2, pp. 33–90, Taf. 1–10, 1882.

Idem. "System und Morphologie der Oligochaeten," Prag, 1884.

Idem. "Studien über Gordiiden," in 'Zeits. Wiss. Zool.,' Bd. 46, pp. 188–216, Taf. 18, 1888.

Idem. "Entwickelungsgeschichtliche Untersuchungen," Prag, 1892.

VIGNAL, W. "Recherches histologiques sur les centres nerveux de quelques Invertébrés," in 'Arch. Zool. Exper.,' Sér. 2, T. 1, pp. 267–412, Plates 15–18, 1883.

VON WAGNER, F. "Beiträge zur Kenntnis der Reparationsprozesse bei *Lumbriculus variegatus*, Gr. . II (Schluss) Teil," in 'Zool. Jahrb.,' Abt. Anat., Bd. 22, pp. 41–156, Taf. 3–7, 1905.

WAWRZIK, E. "Ueber das Stützgewebe des Nervensystems der Chaetopoden," in 'Zool. Beiträge,' Bd. 3, pp. 107–127, Taf. 14–19, 1892.

WILLEM, V. "Observations sur l'excrétion chez l'Arénicole," in 'Miscell. Biol. dédiées au Prof. Giard,' pp. 555–576, Plates 29, 30, Paris, 1899.

WILLEM, V., and MINNE, A. "Recherches sur l'excrétion chez quelques Annélides," in 'Mém. Cour. Acad. Roy. Belgique,' T. 58, No. 1, 1900.

WILSON, E. B. "The Embryology of the Earthworm," in 'Journ. Morphology,' vol. 3, pp. 387–462, Plates 16–22, 1889.

7. DESCRIPTION OF THE PLATES.

KEY TO THE REFERENCE LETTERS.

AX. Axone (axis cylinder) of giant fibre.
BR. Branch of giant fibre.
CG. Coagulum (in vacuole).
ENT.F. Fibril entering giant cell.
F. Fibrous portion of nerve cord.
GANG.C. Ganglion cell.
G.C. Giant cell.
G.C. I.....G.C. XVIII. The giant cells numbered consecutively, the most anterior one being G.C. I.
G.F. Giant fibre.
GL.F. Fibril, probably glial.
G.N. General network of neurofibrillæ in giant cell.
GR. Granules.
N. Nucleus.
NFB. Neurofibrillæ.

NFB.AX. Neurofibrillæ of the axone.
NG. Neuroglia.
NL. Nucleolus.
N.M. Nuclear membrane.
P.N.N. Perinuclear network of neurofibrillæ in giant cell.
P.N.Z. Perinuclear zone of protoplasm.
P.Z. Peripheral zone of protoplasm.
S. Sinus.
SH. Sheath of giant cell.
SH.G.F. Sheath of giant fibre.
SH.N. Nucleus of sheath of giant cell.
SH.N.C. Sheath of nerve cord.
SH.N.G.F. Nucleus of sheath of giant fibre.
S.IF. Interfibrillar substance.
S.PF. Perifibrillar substance.
VAC. Vacuole.
I, II, III. Giant fibres the numbers of which correspond with the number of the cell from which each fibre arises.

Note on the Methods of Staining of the Preparations figured.

The giant cells shown in fig. 17 are drawn from living or fresh specimens, all the other figures on Plates 32, 33, 34, 35, and 37 are from sections of specimens preserved in sublimate-acetic mixture, except figs. 20, 22–29, 40, 55, 56, which are from material preserved and treated by BETHE'S method (see p. 476). All the sections are stained with iron-hæmatoxylin, except the one from which fig. 40 was drawn, which was stained by PRENTISS' modification of BETHE'S method. The mode of staining of the sections shown on Plate 36 is indicated in the description of each figure.

In all the figures (except fig. 41) of sections of giant cells the lower side of the figure is ventral.

PLATE 32.

Halla parthenopeia.

The figures on this plate (and figs. 9–15 on Plate 33) are all drawn from one specimen, about 30 inches in length when living, to show the whole series of giant cells and the course of the giant fibres in the first eight segments. The giant cells are numbered consecutively (G.C. I to G.C. XVIII), and their giant fibres bear corresponding numbers (I to XVIII). Each of the figures is composite, that is, does not represent a single section, but is formed of the superposed drawings of several successive sections. Each of the first two figures represents a giant cell and its giant fibre as seen in two and in three successive sections respectively. Each of the other figures is built up from a larger number of sections, from fifteen to sixty; most of the

figures thus represent slices of considerable thickness, as is diagrammatically represented by the dorsal side of each slice (except in figs. 1 and 2) being shown in perspective. The antero-posterior thickness of each slice represented is indicated to the left of the plan of this nerve cord shown in fig. G (p. 450). The cellular part of the cord is the darker area in the ventral part of each section, the fibrous part is the lighter dorsal portion. The sheath of the nerve cord is shown only in figs. 1 and 2; the sheath of the giant fibres is not separately indicated until the fibres begin to run in a longitudinal direction. The observer is supposed to be at the anterior end of the animal and looking posteriorly along the nerve cord. The parts in each section near to the observer (on or near the cut surface of the section) are shown in light tone, those further away are shaded. All the sections were drawn by means of a camera lucida and are magnified 150 diameters.

Fig. 1.—Section (20 μ thick) through the œsophageal connectives just before they unite with the ventral nerve cord, showing the first giant cell (G.C. I)—a secondary cell. The giant fibre could only be traced as far as it is shown; its sheath then becomes very thin, and the fibre is, for a time, lost to view among the ordinary nerve fibres. It reappears near the mid-dorsal line in fig. 4. The thick neurilemma sheath (Sh.N.C.) which invests the connectives and the nerve cord is shown in this and the following figure only.

Fig. 2.—Section (30 μ thick) through the anterior end of the nerve cord, just behind the point of union of the œsophageal connectives, showing the second giant cell (G.C. II)—a secondary cell. The sheath of its giant fibre becomes very thin, and the fibre is temporarily lost to view among the ordinary nerve fibres. It reappears near the mid-dorsal line in fig. 4.

Fig. 3.—Section (200 μ thick) showing the third and fourth (secondary) giant cells and their fibres crossing the cord; the giant fibre on the left gives off a branch (Br.) into the fibrous portion of the cord.

Fig. 4.—Section (350 μ thick) showing the fifth and sixth giant cells—the first couple of primary giant cells. The giant fibres (I, II) from the first and second cells are seen near the mid-dorsal line, those from the third and fourth (III, IV) are tending mid-dorsally.

Fig. 5.—Section (240 μ thick) showing a lateral secondary giant cell (G.C. VII): its fibre was lost to view among the ordinary nerve fibres at the point marked VII; the same fibre probably reappears near the mid-dorsal line in fig. 7. The fibre (II) from the second giant cell disappears in this section and is not subsequently met with.

Fig. 6.—Section (380 μ thick) showing the eighth and ninth giant cells—the second couple of primary giant cells—one of which is situated in the lateral cell group.

Fig. 7.—Section (300 μ thick) showing the tenth and eleventh giant cells—the third segmental couple. The eleventh is almost hidden behind the tenth, both cells being median. The giant fibre (I) from the first giant cell disappears in this section and is not subsequently recognisable. A giant fibre, probably from G.C. VII, appears in this section near the mid-dorsal line.

Fig. 8.—Section (500 μ thick) immediately posterior to the preceding, showing the continuations of the various giant fibres III to XI.

Plate 33.

Halla parthenopeia.

Figs. 9 to 15 form a continuation of the series shown on Plate 32. See the general remarks upon these figures above (p. 512).

Fig. 9.—Section (280 μ thick) showing the twelfth and thirteenth giant cells—the fourth segmental couple. The posterior cell is almost hidden behind the anterior one.

Fig. 10.—Section (520 μ thick) immediately posterior to the preceding, showing the continuations of the giant fibres.

Fig. 11.—Section (600 μ thick) showing the fourteenth and fifteenth giant cells—the fifth segmental couple. The giant fibres III to XI have now attained their final position in the dorsal region of the cord. The fibres from the giant cells numbered XII to XVII run near the middle, or in the ventral, portion of the fibrous part of the cord, and do not tend dorsally, at any rate until they have traversed a considerable portion of the cord, as may be seen from the four following figures.

Fig. 12.—Section (340 μ thick) showing the sixteenth and seventeenth giant cells—the sixth segmental couple.

Fig. 13.—Section (400 μ thick) immediately behind the one drawn in fig. 12, to show the continuations of the giant fibres. Note also the increase in calibre of some of the giant fibres (compare with previous sections).

Fig. 14.—Section (500 μ thick) showing the last giant cell—the single primary giant cell of the seventh segment. Its fibre could only be followed a comparatively short distance, and was then, owing to thinning of its sheath, lost among the ordinary nerve fibres.

Fig. 15.—Section (150 μ thick) through the anterior part of the ninth segment, showing eight giant fibres running along the dorsal portion of the cord and six near the middle or in the ventral portion of the fibrous part of the cord. One of the giant fibres (X) gives off a branch which crosses the cord and runs longitudinally.

Fig. 16.—Section through the fourth giant cell of the specimen, 44 mm. long. Note.—The stout glia sheath of the cell continued, but in diminished amount, on to the giant fibre; the granular vacuolated cytoplasm of the giant cell not divided into general and peripheral portions (*cf.* fig. 18); the nucleus with its enveloping perinuclear zone of cytoplasm. The giant fibre (G.F. III) of the third giant cell is seen in the left portion of the fibrous part of the cord; the giant fibres from the first and second cells are not recognisable. × 430.

Plate 34.

Halla parthenopeia.

Fig. 17.—Optical sections of four cells drawn from living or fresh specimens to show the granules, yellow in life, which form one or more masses in the cell. Surrounding the nucleus is a specialised zone of protoplasm—the perinuclear zone (P.N.Z.)—limited externally by the perinuclear network of neurofibrillæ, which can be clearly seen in the fresh condition; it appears as seen under this magnification as a line (P.N.N.). The peripheral portion of the protoplasm (P.Z.) is pale and non-granular; the general protoplasm contains fine granules, not shown in the figure, smaller than the yellow granules. The sheath of two of the giant cells is shown. × 120.

Fig. 18.—Section of a small giant cell—the fourth cell of the specimen from which fig. 3 (G.C. IV) and the plan shown on p. 450 were drawn. The cell clearly shows the perinuclear, general, and peripheral regions of the protoplasm. Fine fibrils, probably glial, pass from the sheath into the peripheral zone of protoplasm. The perinuclear network of fibrillæ (P.N.N.) is shown, but the general network is not drawn, as it is obscured in the cell by the numerous protoplasmic granules. The details of the sheath are not given, and only about one-third of the thickness of the sheath is represented. × 1000.

Fig. 19.—Section of the nucleus and perinuclear zone of a medium-sized giant cell showing the chromatin granules and achromatic strands of the nucleus, the "double nucleolus" (Nl.), the chromophilous granules and the vacuoles of the perinuclear zone of protoplasm, and the perinuclear network of neurofibrillæ (P.N.N.). × 1000.

Fig. 20.—"Double nucleoli" from three giant cells. × 1000.

Fig. 21.—Section of a portion of the peripheral region of a small giant cell in which numerous fibrils, probably of glial nature, are seen passing from the sheath into the peripheral zone of protoplasm, where they branch and interlace. × 1000.

Figs. 22, 23.—Two consecutive sections (each 8 μ thick) of a small giant cell, to show the neurofibrillar network.

Fig. 22 is from a section passing through the nucleus and perinuclear zone. At the margin of the latter is the perinuclear network, continuous on its outer aspect with the general network, which in turn is continuous with the fibrillæ which pass out through the axone. The sheath is too darkly stained to permit details of its structure to be made out. × 1000.

Fig. 23 is a tangential section in which only a small part of the nucleus is seen. The strands of the perinuclear network are distinguished by their slightly greater thickness from those of the general network. × 1000.

Fig. 24.—Section of a large giant cell to show the perinuclear and general network of neurofibrillæ. The origins of the axone fibrillæ do not lie in the plane of this section, but in neighbouring sections these fibrillæ may be traced into connection with the general network. Around the margin of the cell numerous short fibrils—probably glia fibrils (GL.F.)—may be seen passing into the peripheral zone of protoplasm of the cell. Longer entrant fibrils (ENT.F.) appear to join the general network of neurofibrillæ (see pp. 481–483). The perinuclear zone of protoplasm in this cell is extremely narrow; its granules are not shown. Note also the "double nucleolus." × 600.

Fig. 25.—Section of a large giant cell passing tangentially through the perinuclear network to show the stouter strands and closer meshes of this network as compared with the general network. The axone and its fibrillæ are also cut tangentially. Several long fibrils (ENT.F.) enter the cell and apparently become continuous with the neurofibrillar network (see pp. 481–483). Shorter, probably glial, fibrils are also present at the periphery of the cell. Only the inner portion of the sheath of the cell is indicated. × 600.

Fig. 26.—A portion of the perinuclear network of the same cell to show the different levels of the constituent meshes; those nearer the observer are drawn in darker lines. × 1000.

Figs. 27, 28.—Two consecutive sections (8 μ thick) of the nerve cord to show branches of the giant fibres. Nine giant fibres run along the dorsal region of this part of the cord and five run in the middle or ventral portion of the fibrous part of the cord. Three of the dorsal and one of the ventral ones give off branches. The branches of the former fork and the twigs pass into the right and left portions of the neuropile, being lost to view among the ordinary nerve fibres as soon as their sheath disappears (but see pp. 473, 487). BR.$^+$ on fig. 27 is continuous with BR.$^+$ on fig. 28. A portion of BR.$^+$ on fig. 28 is shown more highly magnified in fig. 29. The details of the fibrous part (F.) of the cord are too minute and complex to

be represented on this scale of magnification; this area, left white in the figure, consists of interlacing neuroglia fibres and very numerous transverse and longitudinal neurofibrillæ. × 180.

Fig. 29.—A portion of the branch indicated by Br.+ on fig. 28, to show the thin sheath, the neurofibrillæ, and the perifibrillar and interfibrillar substance. × 1200.

Plate 35.

Figs. 30–36.—*Aglaurides fulgida.*

Fig. 30.—Section of a large giant cell. The perinuclear network is cut tangentially in its upper portion. Each of the axone fibrillæ is formed by the fusion of a number of primitive fibrillæ which are in connection with the general network. This latter, which is composed of very slender strands, is visible over a portion only of the cell. Several long entrant fibrils are seen right and left of the axone. The perinuclear zone is much more oval in shape than the nucleus, and over the greater portion of the nucleus forms only a very thin envelope; the granules of this zone and of the general cytoplasm are not represented (for these, see fig. 32). The large and small nucleoli are added from the next section. × 750.

Fig. 31.—A somewhat tangential section of a medium-sized giant cell. The general network is here differentiated in a zonary manner, there being a zone in which the fibrils are distinctly stouter than the rest and resemble those of the perinuclear network. Sinuses (S.) enter the cell in its lower (ventral) portion accompanied by clusters of fibrillæ, some, at least, of which are glial. Longer entrant fibrils are seen, especially near the axone, some of which appear to be continuous with the neurofibrillar network. × 1000.

Fig. 32.—Section of a giant cell similar to the preceding to show the structure of the nucleus and cytoplasm. The peripheral zone of protoplasm is very feebly differentiated and only over a portion of the cell (*cf.* fig. 18). A darkly stained area extends from the upper end of the nucleus into the cone of origin of the axone. The perinuclear zone of protoplasm and the perinuclear network are indicated, as also are the fibrillæ in the axone; but the general network is not shown, being obscured by the cytoplasmic granules. × 600.

Fig. 33.—Tangential section of a giant cell showing sinuses in the peripheral portion of the protoplasm of the cell. × 300.

Fig. 34.—Vertical section of a large giant fibre showing the sinuous bundle of neurofibrillæ. There are altogether about sixteen fibrils in this bundle, but only seven of these, which are seen on focussing the upper edge of the bundle, are drawn. Two of the fibrillæ are distinctly stouter than the rest. Between the fibrillæ there is a faintly stained, almost homo-

geneous interfibrillar substance; between the bundle of fibrillæ and the sheath of the giant fibre lies the highly vacuolated, finely granular, perifibrillar substance. × 1400.

Fig. 35.—Vertical section of another giant fibre showing the sinuous bundle of neurofibrillæ from which a fibril crosses the lumen of the fibre to pass out through the sheath. × 1000.

Fig. 36.—Transverse section of the mid-dorsal region of the nerve cord showing two large and two small giant fibres. In each of the two large fibres there are eighteen neurofibrillæ, the cut ends of which are seen lying in the interfibrillar matrix; around this is the vacuolated perifibrillar substance. In each of the two smaller giant fibres there are apparently only two neurofibrillæ. × 600.

Figs. 37–40.—*Halla parthenopeia.*

Figs. 37, 38, 39.—Horizontal sections of a medium-sized giant fibre at three different levels.

Fig. 37.—Tangential section through the outer portion of the sheath, to show that this portion of the sheath is composed of stout, chiefly longitudinal, glia fibrils. Three glia nuclei are also seen. × 1000.

Fig. 38.—Tangential section through the inner portion of the sheath to show the more slender interlacing glia fibrils which form this part of the sheath. The granular remains of the glia protoplasm and two glia nuclei are also shown. × 1000.

Fig. 39.—Central section of the giant fibre showing the slender bundle of neurofibrillæ, the interfibrillar (S.If.), and perifibrillar (S.Pf.) substance. The neurofibrillæ are almost straight (*cf.* figs. 34, 35), as the specimen from which they are drawn was killed fully extended. There are altogether eight to ten fibrillæ in this bundle, but only those are drawn which are seen on focussing the upper edge of the bundle; the other four to six fibrillæ lie at a deeper level. The bundle of fibrillæ is very slender in this fibre, probably owing to the undue contraction of the interfibrillar substance on fixation. × 1000.

Fig. 40.—Horizontal section of a giant fibre. The fibrillæ are very thin and sinuous. The details of the sheath are not shown. × 1200.

PLATE 36.

Halla parthenopeia.

The figures on this plate were drawn, from preparations by the author, by Mr. RICHARD MUIR.

Fig. 41.—Horizontal section of two large adjacent giant cells—a segmental couple. The section from which these are drawn was cut by a freezing microtome

from a specimen recently preserved in formalin, and was then stained with Sudan III. The granules upon which this stain and Scharlach R exercise a selective action are shown. They are yellow in the living cell (see fig. 17). The lower cell shows also the nucleus, the perinuclear zone, and numerous fibrils, probably glial, passing from the sheath into the peripheral zone. × 300.

Fig. 42.—A large ganglion cell from the same section as the preceding, showing the granules in the protoplasm, stained with Sudan, chiefly at the end of the cell opposite the axone. × 1000.

Fig. 43.—A medium-sized ganglion cell from the same section as the two preceding figures, showing the granules, stained with Sudan, at the end of the cell opposite the axone. The granules shown in this and in the preceding cell are yellow in life. × 1000.

Fig. 44.—Three small chlorogogen cells from the cellular part of the nerve cord. The section from which these were drawn was cut by a freezing microtome from a specimen recently preserved in formalin, and was then stained successively with Scharlach R and with hæmatein. The cells are full of red-stained granules, most of which were yellow in life. × 1000.

Fig. 45.—Section of a large giant cell stained with methylene blue to show the chromophilous perinuclear zone of protoplasm (the portion of which applied to the nuclear membrane is especially dense), the granular general cytoplasm, and the non-granular peripheral cytoplasm. Vacuoles are present in the perinuclear and general cytoplasm. This method of staining does not show the fibrils of the sheath of the cell, but the glia nuclei of this sheath are well seen. × 500.

Fig. 46.—Section of a small giant cell stained with methylene blue. The perinuclear zone is not so clearly differentiated as in the preceding cell, but the peripheral zone is clearly marked. The cone of origin of the axone is non-granular. × 500.

Fig. 47.—Section of a large giant cell stained with toluidin blue and erythrosin. The nuclear membrane and chromatin are acidophile, the granules in the perinuclear zone are basophile, as are most of those in the general cytoplasm. The peripheral zone is stained pink, and the granules therein present are sparse and minute. In some of the vacuoles in the perinuclear and general cytoplasm there is a red-stained coagulum which is granular in some cases. The perinuclear zone in this cell is very narrow and its margin entire (*cf.* fig. 48). Note also the "double nucleolus." × 500.

Fig. 48.—Section of a large giant cell stained with toluidin blue and erythrosin. The perinuclear zone is broader than in the preceding cell and at one end there are strands which extend from it into the general cytoplasm. × 500.

Fig. 49.—Section of a small giant cell stained with toluidin blue and erythrosin showing the protoplasm differentiated into the three well-marked regions—perinuclear, general, and peripheral. The nuclear membrane and chromatin are acidophile, the granules of the perinuclear and most of those of the general cytoplasm are basophile. The peripheral zone and axone are stained faintly blue and are non-granular. × 500.

Fig. 50.—Medium sized giant cell. This and the one drawn in fig. 47 are from the same worm and are stained in the same way, that is, with toluidin blue and erythrosin. The cytoplasm appears, at first sight, to be almost homogeneous, but contains faintly staining granules which are less basophile than usual. There is no definite perinuclear zone and no peripheral zone of protoplasm is differentiated. The nuclear membrane and chromatin are acidophile, as usual. Note also the "double nucleolus." × 500.

Fig. 51.—"Double nucleoli" from three giant cells stained with toluidin blue and erythrosin. Each nucleolus contains acidophile bodies enveloped by a basophile substance. × 1000.

Plate 37.

For the figures on this Plate, which are from untouched negatives, I am indebted to the skill of Mr. Max Poser (figs. 54–59) and Mr. Richard Muir (figs. 52, 53).

Figs. 52–56.—*Halla parthenopeia.*

Fig. 52.—Vertical section through the ventral body wall and nerve cord. The left side of the section is anterior. The third and fourth and a portion of the second chætigerous segments are included. The section shows the second, third, and fourth couples of primary giant cells, which are so situated that the posterior cell of each couple lies at the posterior end of the segment. The first part of the axone of the second cell from the left is seen. The fourth cell from the left is shown in the following figure. The division of the cord into a dorsal fibrous portion and a ventral cellular portion, the nuclei of which are seen, may be noted. Dorsal to the cord is its thick sheath containing blood-vessels. × 60.

Fig. 53.—The fourth cell from the left in the preceding figure. A large giant cell showing the nucleus, the ovoid perinuclear zone, somewhat more diffuse than usual, and the collection of dark granules in the protoplasm at the anterior (left) end of the cell. The radial lines in the peripheral zone of protoplasm are short glia fibrils. × 480.

Fig. 54.—Another large giant cell from the same specimen showing the perinuclear zone, at the lower margin of which a portion of the perinuclear network is seen. To the right of the upper margin of the perinuclear zone a few

strands of the general neurofibrillar network may be seen. Numerous short faintly-stained radial glia fibrillæ are seen passing into the peripheral zone and, on the left, one longer entrant fibril which may be traced nearly to the perinuclear network. × 550.

Fig. 55.—Section of a small, nearly spherical, giant cell and the first portion of its giant fibre to show the course of the latter and its central bundle of darkly-stained fibrillæ. Note also the nucleus and, concentric with it, the narrow perinuclear zone. × 350.

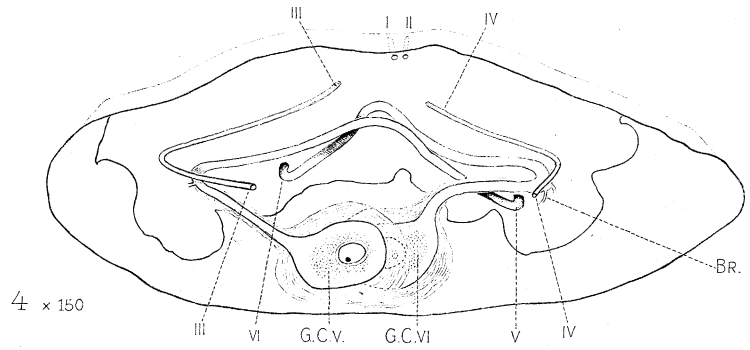
Fig. 56.—Section of a small giant cell showing the nucleus and nucleolus, the perinuclear zone, the perinuclear network and the general network of neurofibrillæ. × 800.

Figs. 57–59.—*Aglaurides fulgida.*

Fig. 57.—Section of a large giant cell and its process to show especially the axone fibrillæ. In the darker cytoplasm to the right of the axone there may be seen slender fibrillæ which have entered through the sheath. The general network, which in this cell is composed of very fine strands, is not seen in the photograph. × 600.

Fig. 58.—The axone and neighbouring portion of the giant cell shown in the preceding figure, showing the axone fibrillæ and, to the right of the latter, entrant fibrils. × 1000.

Fig. 59.—Vertical section of a large giant fibre to show the sinuous bundle of neurofibrillæ lying in the centre of the lumen of the fibre. The sheath of the fibre is clearly seen in the lower (ventral) part of the photograph. Dorsal to the fibrillæ, between them and the sheath of the giant fibre, the vacuolated remains of the perifibrillar substance may be seen. × 1000.



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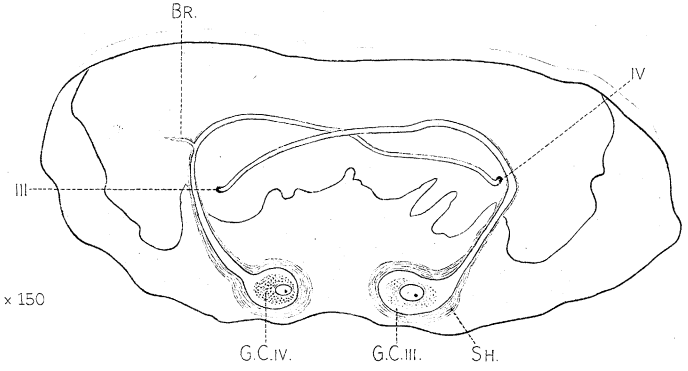

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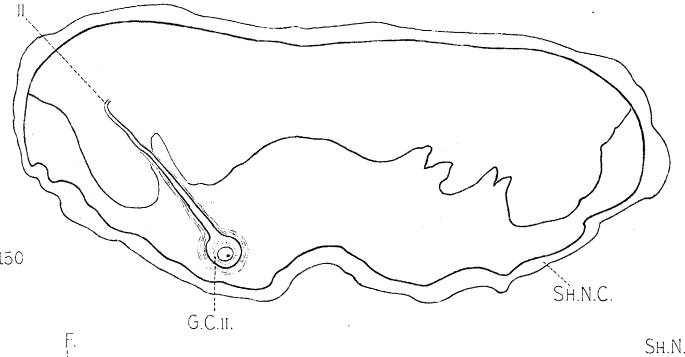

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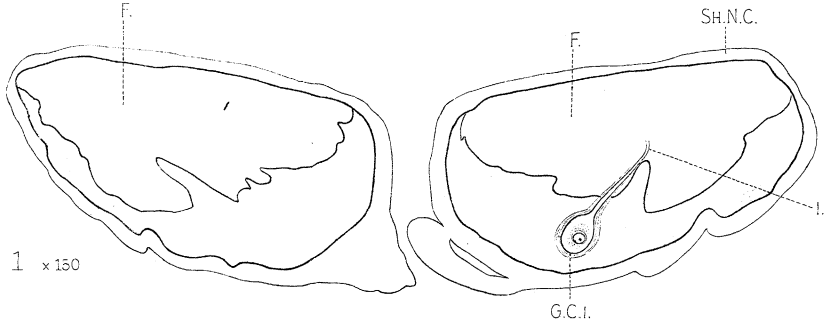

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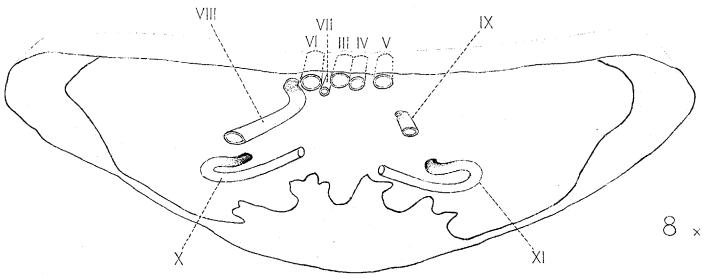

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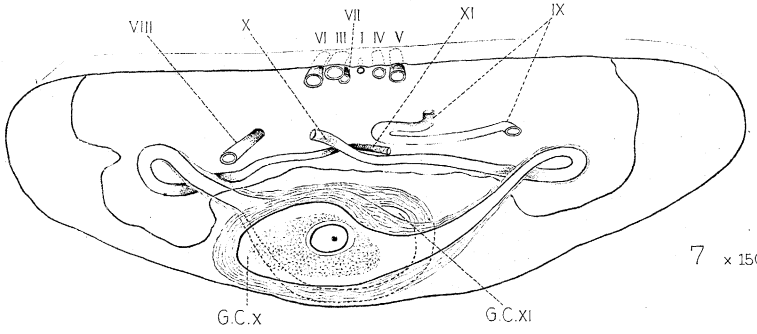

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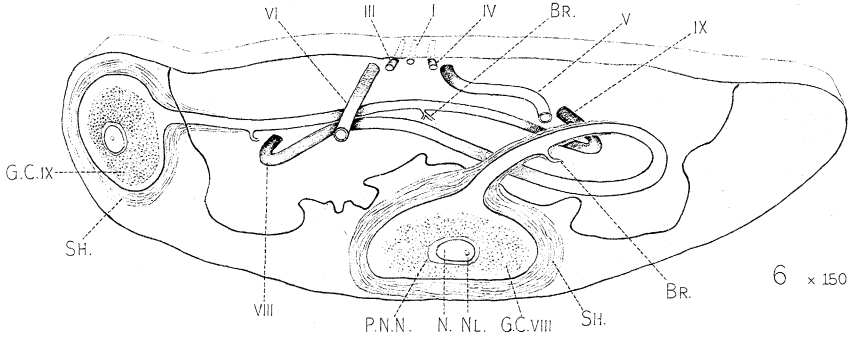

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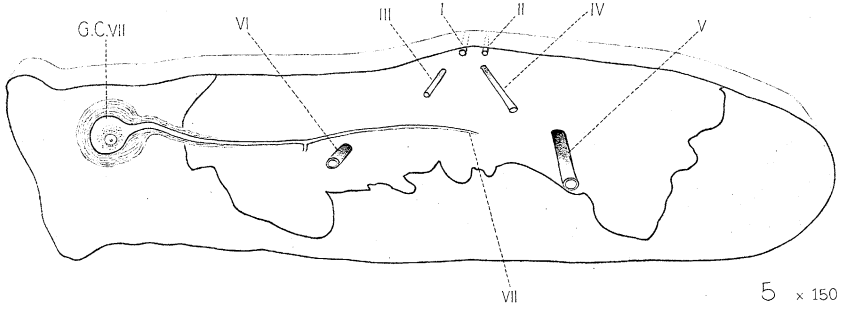

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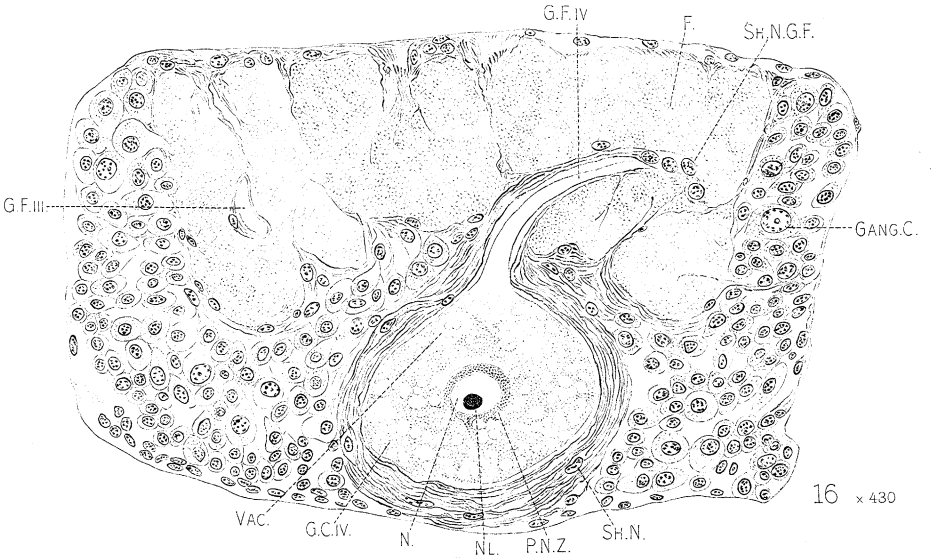

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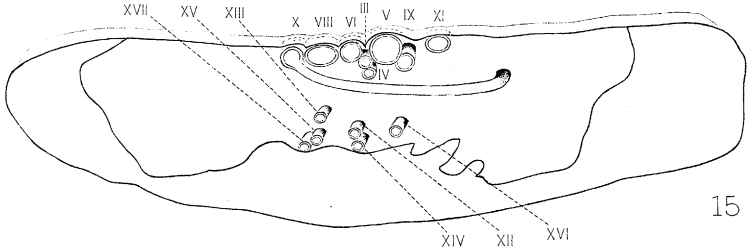

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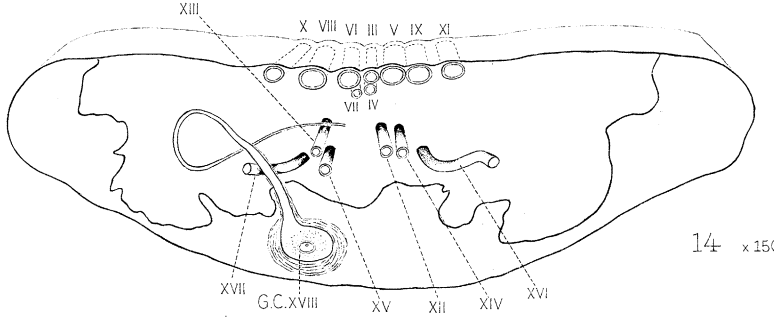

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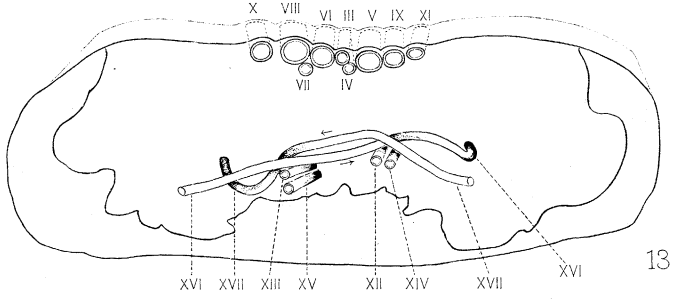

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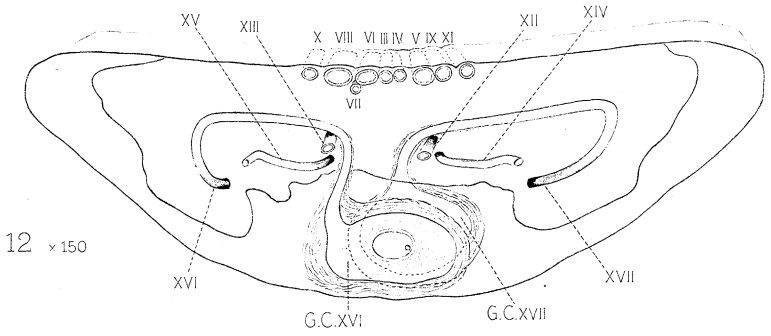

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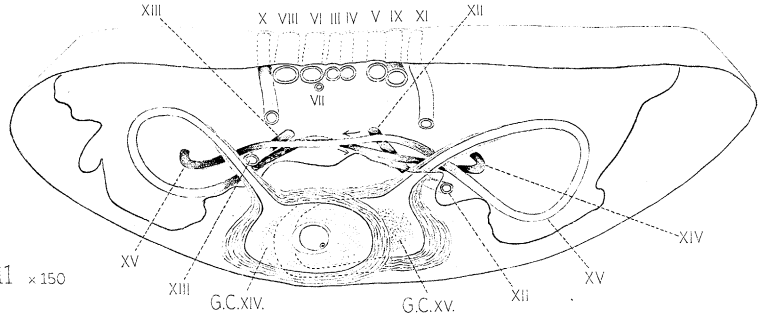

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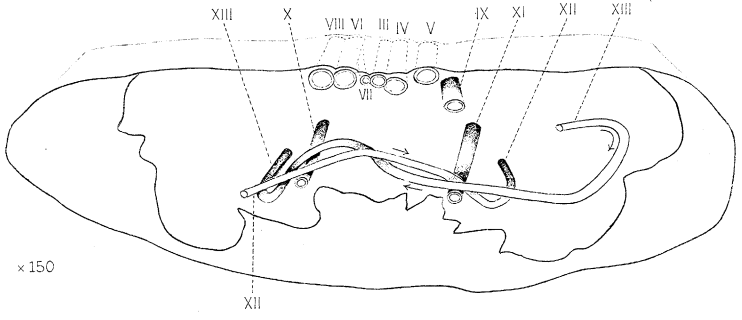

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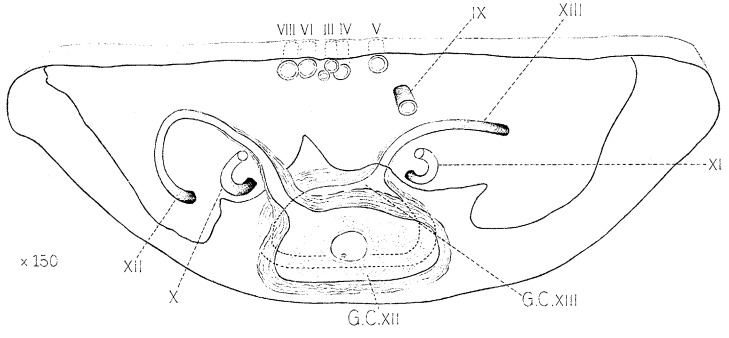

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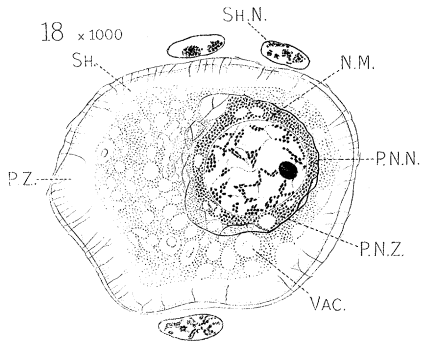
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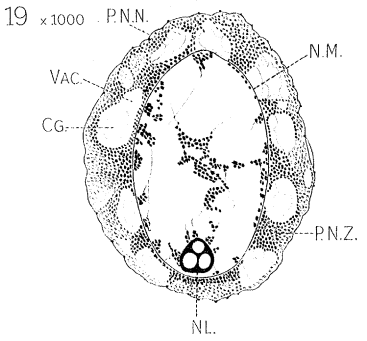
P.N.N.
P.Z.
P.N.Z.
VAC.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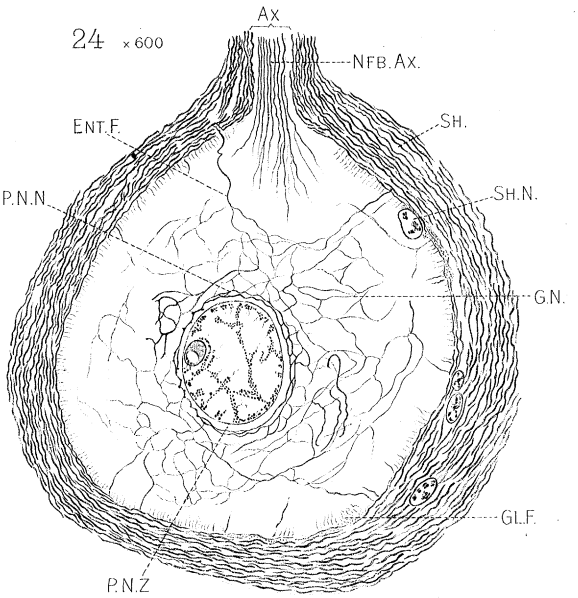
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GL.F.
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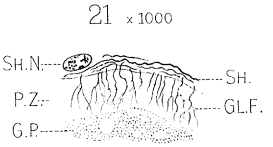
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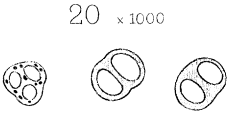
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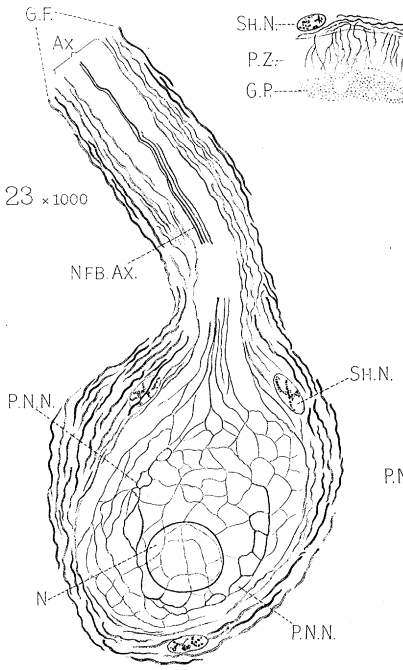
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AX.
23 x 1000
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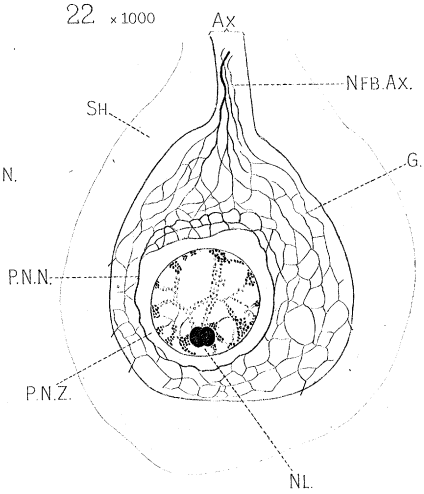
SH.N.
P.N.N.
N.
P.N.N.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SH.
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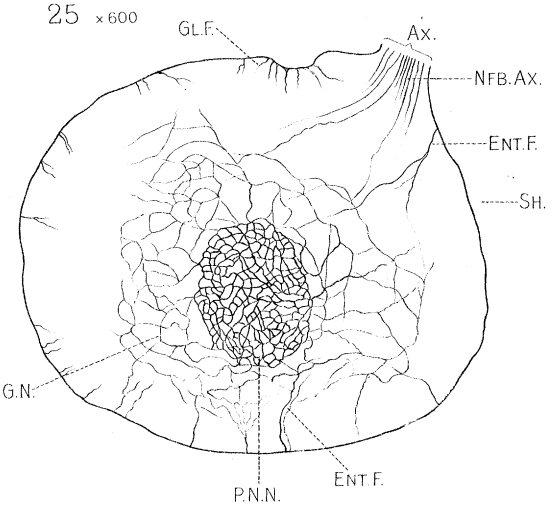
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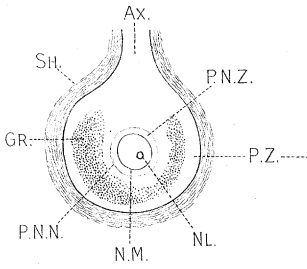
SH.
G.N.
P.N.N.
ENT.F.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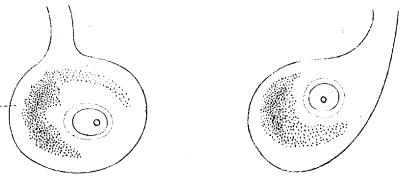
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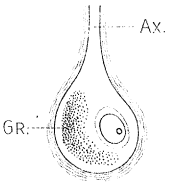
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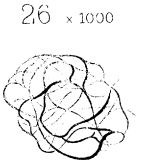
GR.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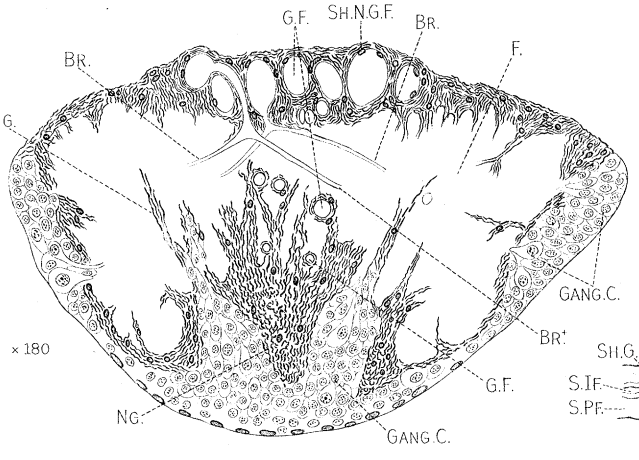
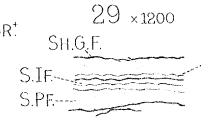

G.F.
SH.N.G.F.
BR.
F.
BR.
NG.
GANG.C.
BR*
G.F.
NG.
GANG.C.
27 x 180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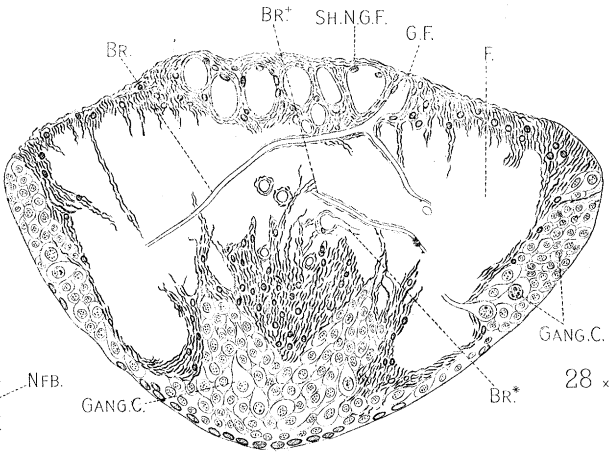
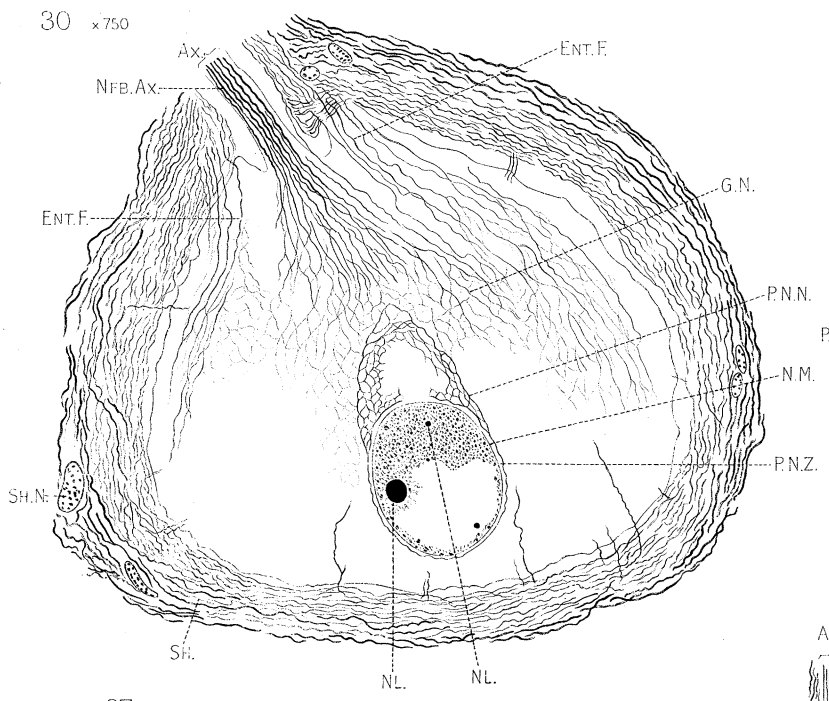
NFB.
S.IF.
S.PF.


BR+
SH.N.G.F.
G.F.
BR.
F.
GANG.C.
GANG.C.
BR*
28 x 180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G.N.
P.N.N.
N.M.
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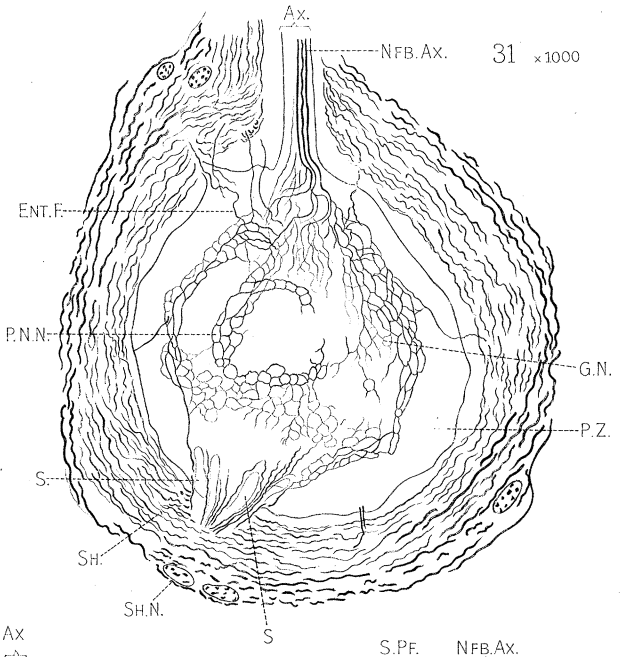
SH.
NL.
NL.


AX.
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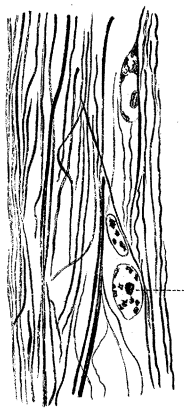
31 ×1000
ENT.F.
P.N.N.
G.N.
P.Z.
S
SH.
SH.N.
S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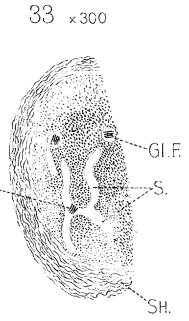
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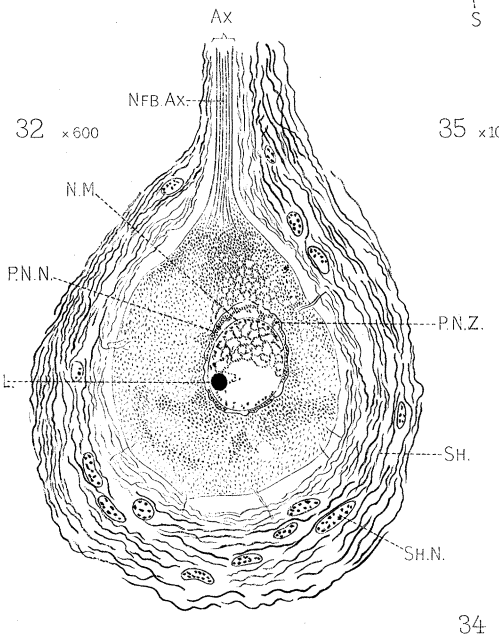
GL.F.
GL.F.
S.
SH.


AX
NFB.AX.
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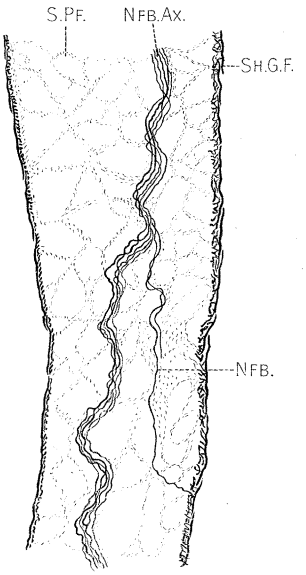
N.M.
P.N.N.
P.N.Z.
NL.
SH.
SH.N.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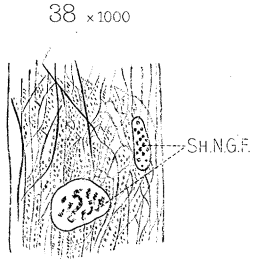
SH.G.F.
NFB.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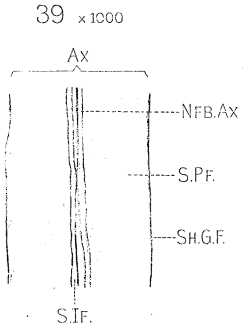
SH.N.G.F.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NFB.AX
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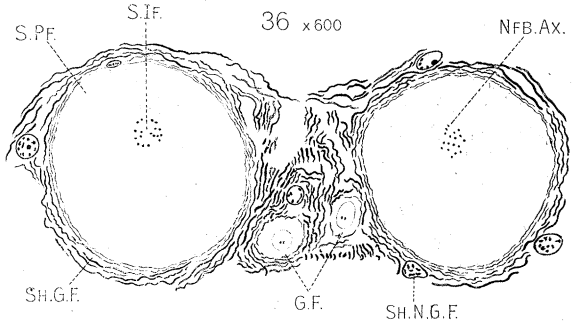
SH.G.F.
S.IF.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S.PF.
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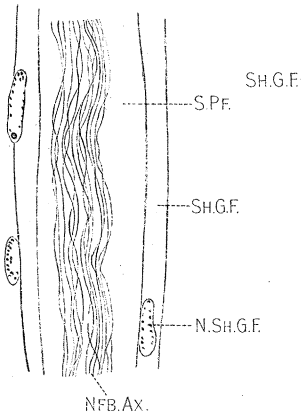
SH.G.F.
G.F.
SH.N.G.F.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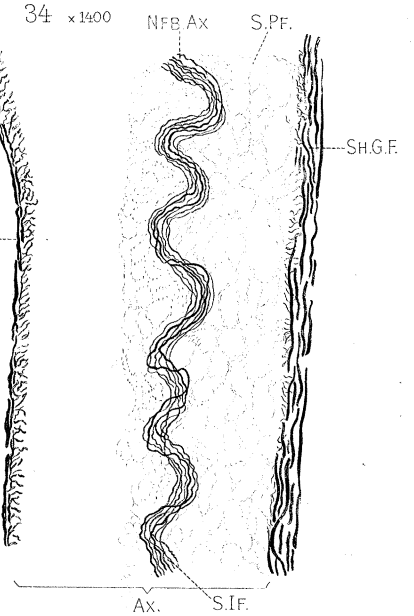
SH.G.F.
N.SH.G.F.
NFB.AX.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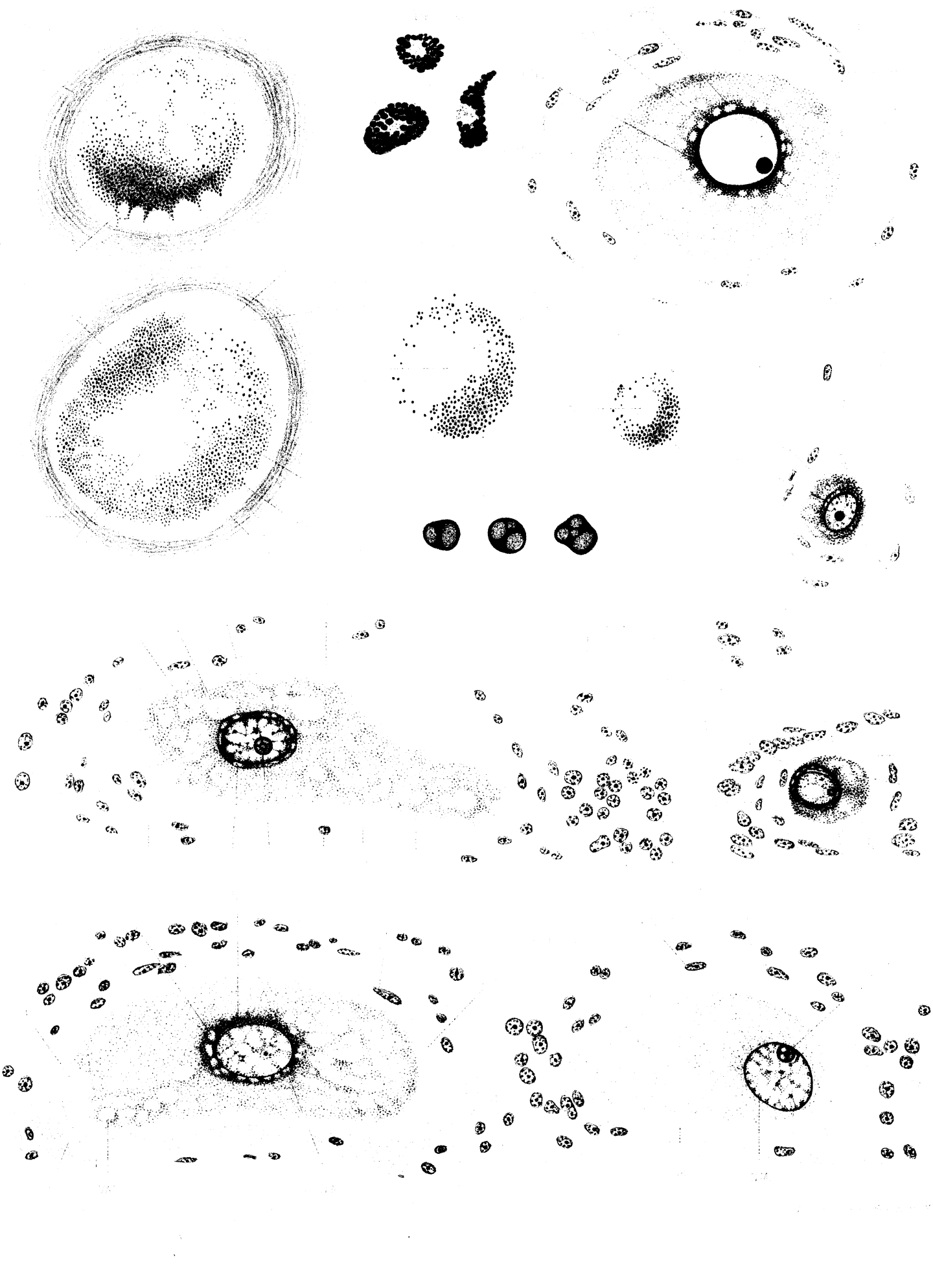
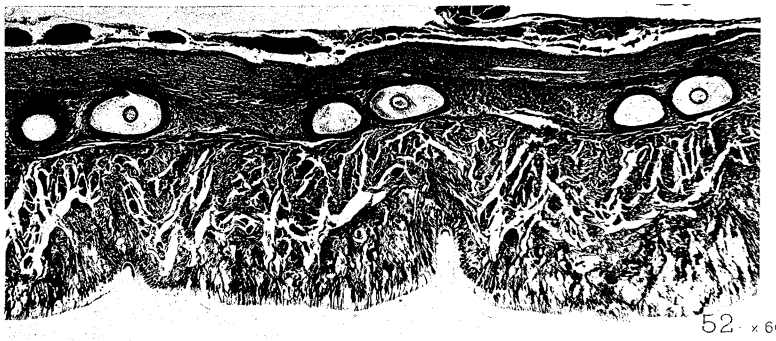
S.PF.
SH.G.F.
SH.G.F.
AX.
S.IF.





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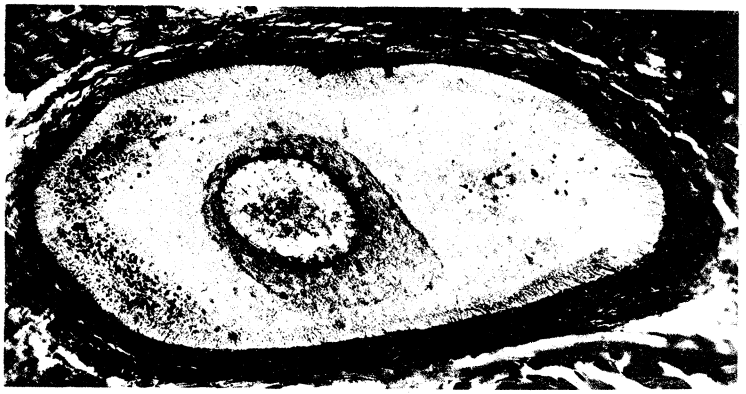
55 × 350



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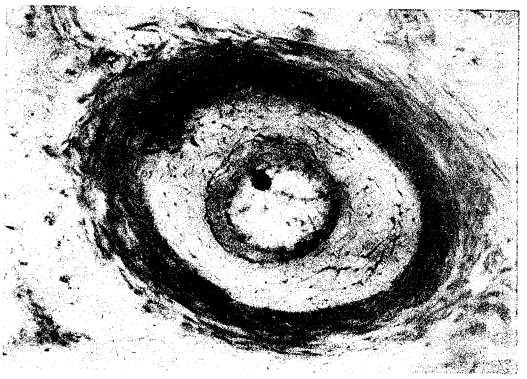

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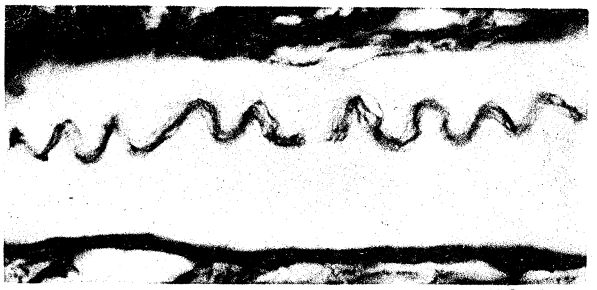
54 × 550



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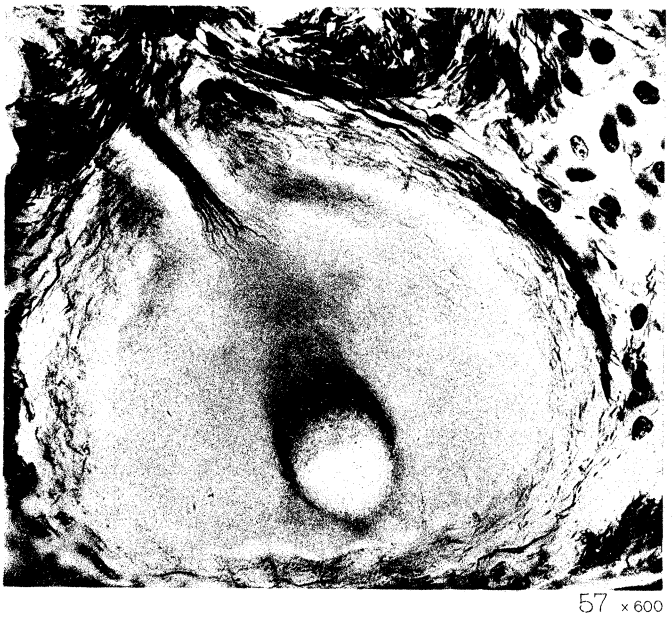


57 × 600

58 × 1000

Werner & Winter, Frankfurt a/M.

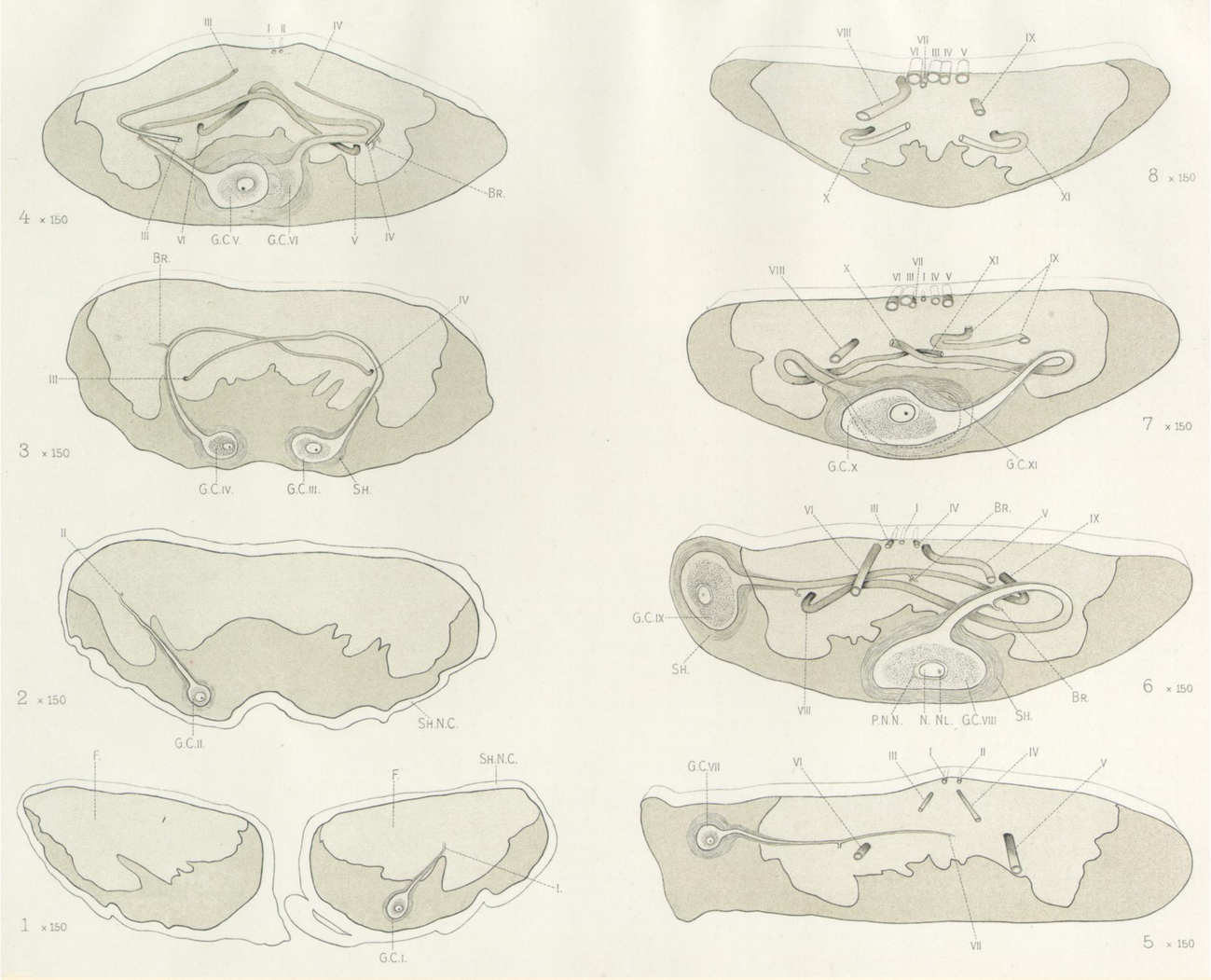


PLATE 32.

Halla parthenopeia.

The figures on this plate (and figs. 9–15 on Plate 33) are all drawn from one specimen, about 30 inches in length when living, to show the whole series of giant cells and the course of the giant fibres in the first eight segments. The giant cells are numbered consecutively (G.C. I to G.C. XVIII), and their giant fibres bear corresponding numbers (I to XVIII). Each of the figures is composite, that is, does not represent a single section, but is formed of the superposed drawings of several successive sections. Each of the first two figures represents a giant cell and its giant fibre as seen in two and in three successive sections respectively. Each of the other figures is built up from a larger number of sections, from fifteen to sixty; most of the figures thus represent slices of considerable thickness, as is diagrammatically represented by the dorsal side of each slice (except in figs. 1 and 2) being shown in perspective. The antero-posterior thickness of each slice represented is indicated to the left of the plan of this nerve cord shown in fig. G (p. 450). The cellular part of the cord is the darker area in the ventral part of each section, the fibrous part is the lighter dorsal portion. The sheath of the nerve cord is shown only in figs. 1 and 2; the sheath of the giant fibres is not separately indicated until the fibres begin to run in a longitudinal direction. The observer is supposed to be at the anterior end of the animal and looking posteriorly along the nerve cord. The parts in each section near to the observer (on or near the cut surface of the section) are shown in light tone, those further away are shaded. All the sections were drawn by means of a camera lucida and are magnified 150 diameters.

Fig. 1.—Section (20 μ thick) through the œsophageal connectives just before they unite with the ventral nerve cord, showing the first giant cell (G.C. I)—a secondary cell. The giant fibre could only be traced as far as it is shown; its sheath then becomes very thin, and the fibre is, for a time, lost to view among the ordinary nerve fibres. It reappears near the mid-dorsal line in fig. 4. The thick neurilemma sheath (SH.N.C.) which invests the connectives and the nerve cord is shown in this and the following figure only.

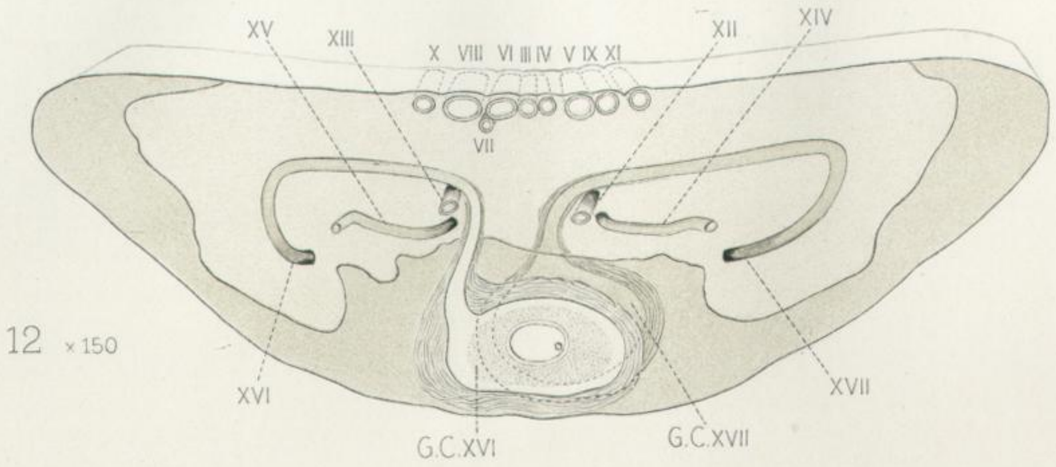
Fig. 2.—Section (30 μ thick) through the anterior end of the nerve cord, just behind the point of union of the œsophageal connectives, showing the second giant cell (G.C. II)—a secondary cell. The sheath of its giant fibre becomes very thin, and the fibre is temporarily lost to view among the ordinary nerve fibres. It reappears near the mid-dorsal line in fig. 4.

Fig. 3.—Section (200 μ thick) showing the third and fourth (secondary) giant cells and their fibres crossing the cord; the giant fibre on the left gives off a branch (BR.) into the fibrous portion of the cord.

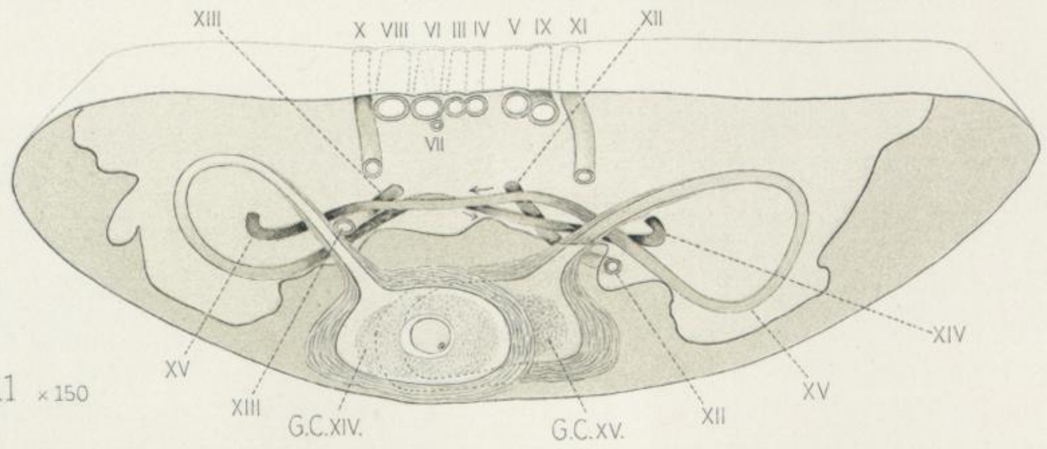
Fig. 4.—Section (350 μ thick) showing the fifth and sixth giant cells—the first couple of primary giant cells. The giant fibres (I, II) from the first and second cells are seen near the mid-dorsal line, those from the third and fourth (III, IV) are tending mid-dorsally.

Fig. 5.—Section (240 μ thick) showing a lateral secondary giant cell (G.C. VII): its fibre was lost to view among the ordinary nerve fibres at the point marked VII; the same fibre probably reappears near the mid-dorsal line in fig. 7. The fibre (II) from the second giant cell disappears in this section and is not subsequently met with.

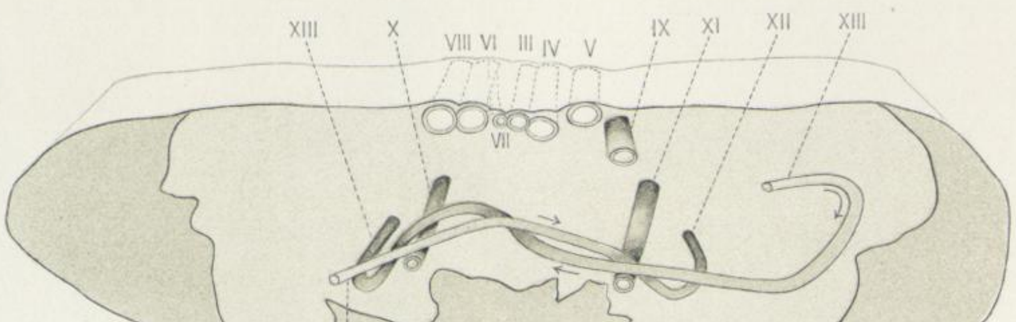
Fig. 6.—Section (380 μ thick) showing the eighth and ninth giant cells—the second couple of primary giant cells—one of which is situated in the lateral cell group.



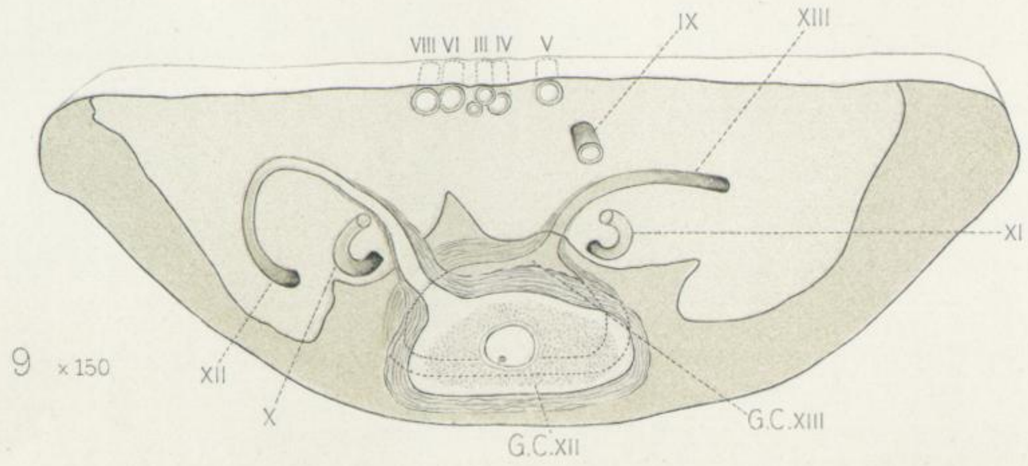
12 × 150



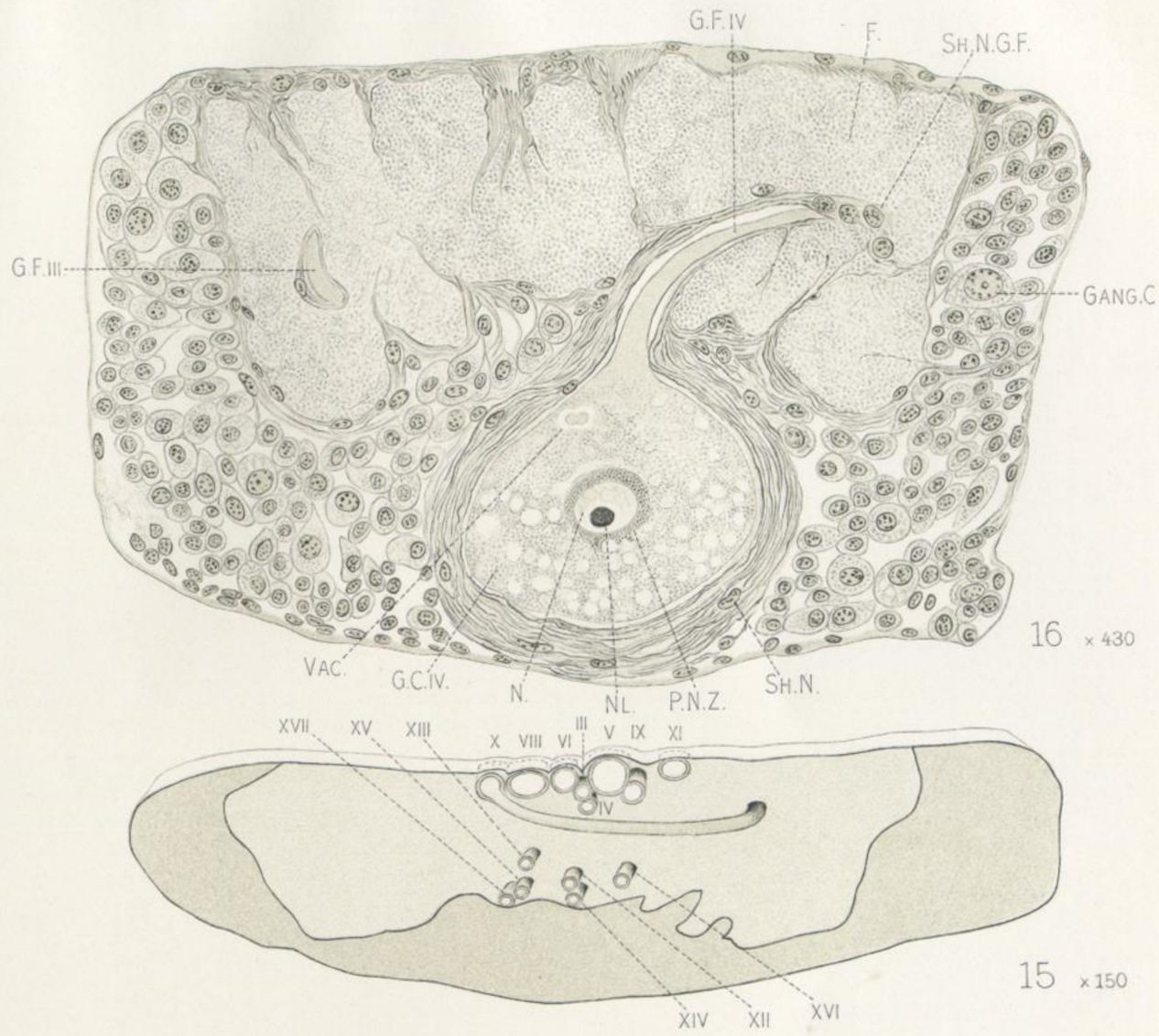
11 × 150



10 × 150

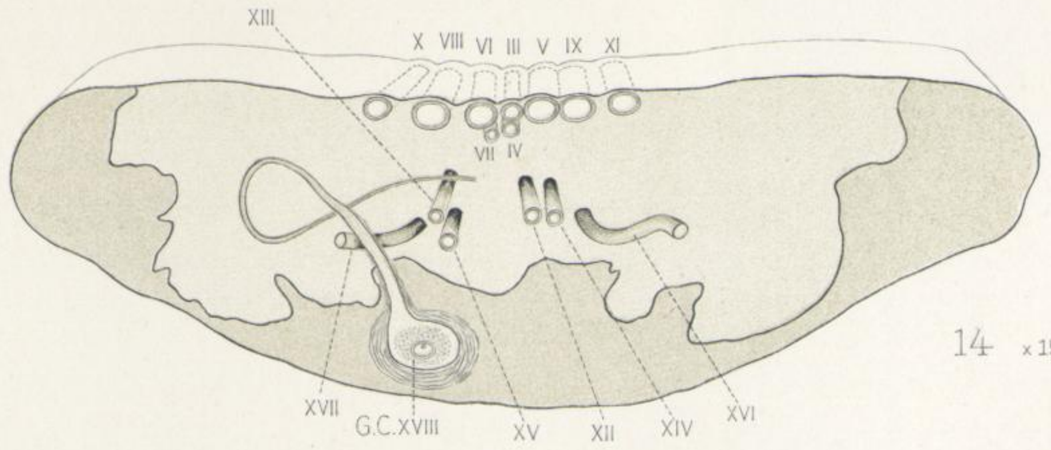


9 × 150

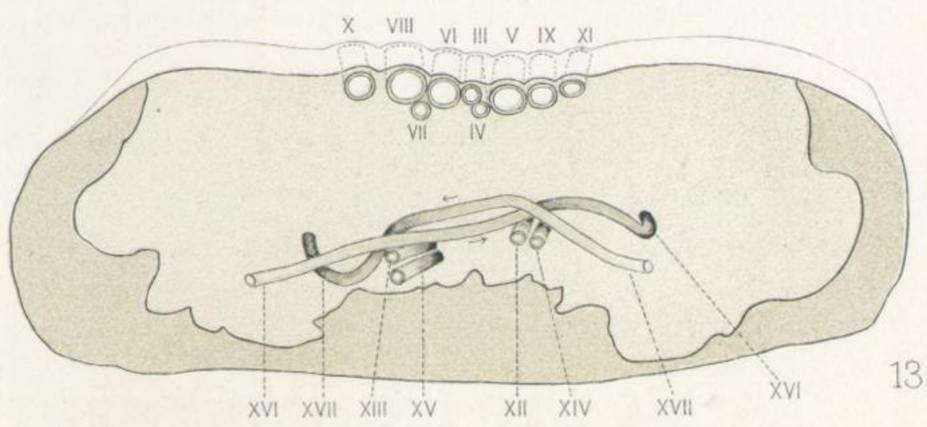


16 × 430

15 × 150



14 × 150



13 × 150

Plate 33.

Halla parthenopeia.

Figs. 9 to 15 form a continuation of the series shown on Plate 32. See the general remarks upon these figures above (p. 512).

Fig. 9.—Section (280 μ thick) showing the twelfth and thirteenth giant cells—the fourth segmental couple. The posterior cell is almost hidden behind the anterior one.

Fig. 10.—Section (520 μ thick) immediately posterior to the preceding, showing the continuations of the giant fibres.

Fig. 11.—Section (600 μ thick) showing the fourteenth and fifteenth giant cells—the fifth segmental couple. The giant fibres III to XI have now attained their final position in the dorsal region of the cord. The fibres from the giant cells numbered XII to XVII run near the middle, or in the ventral, portion of the fibrous part of the cord, and do not tend dorsally, at any rate until they have traversed a considerable portion of the cord, as may be seen from the four following figures.

Fig. 12.—Section (340 μ thick) showing the sixteenth and seventeenth giant cells—the sixth segmental couple.

Fig. 13.—Section (400 μ thick) immediately behind the one drawn in fig. 12, to show the continuations of the giant fibres. Note also the increase in calibre of some of the giant fibres (compare with previous sections).

Fig. 14.—Section (500 μ thick) showing the last giant cell—the single primary giant cell of the seventh segment. Its fibre could only be followed a comparatively short distance, and was then, owing to thinning of its sheath, lost among the ordinary nerve fibres.

Fig. 15.—Section (150 μ thick) through the anterior part of the ninth segment, showing eight giant fibres running along the dorsal portion of the cord and six near the middle or in the ventral portion of the fibrous part of the cord. One of the giant fibres (X) gives off a branch which crosses the cord and runs longitudinally.

Fig. 16.—Section through the fourth giant cell of the specimen, 44 mm. long. Note.—The stout glia sheath of the cell continued, but in diminished amount, on to the giant fibre; the granular vacuolated cytoplasm of the giant cell not divided into general and peripheral portions (*cf.* fig. 18); the nucleus with its enveloping perinuclear zone of cytoplasm. The giant fibre (G.F. III) of the third giant cell is seen in the left portion of the fibrous part of the cord; the giant fibres from the first and second cells are not recognisable. × 430.

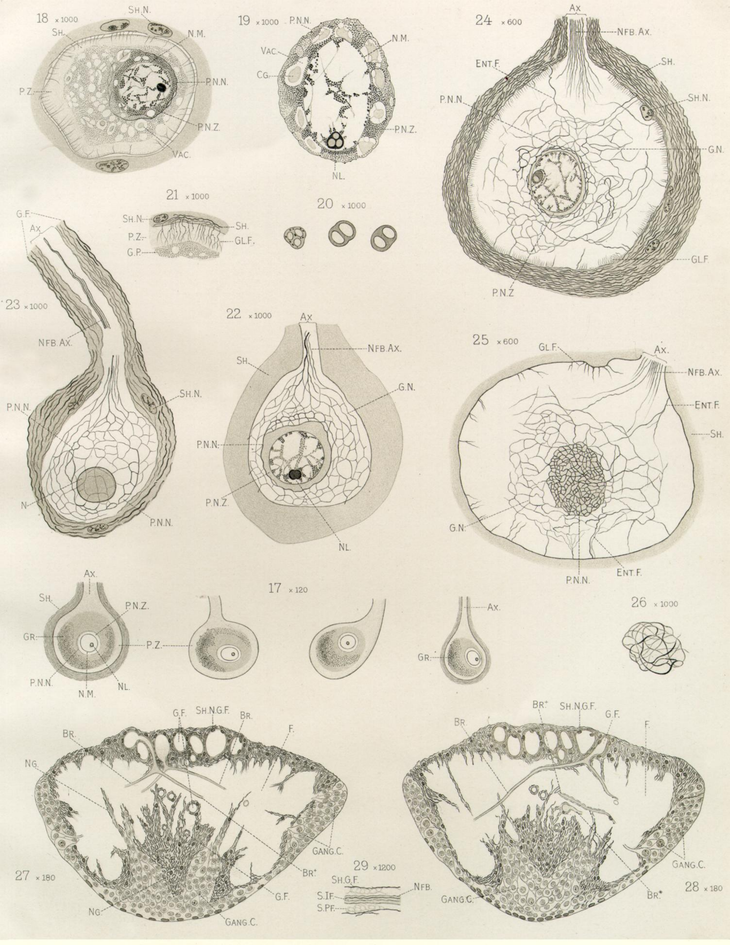


PLATE 34.

Halla parthenopeia.

Fig. 17.—Optical sections of four cells drawn from living or fresh specimens to show the granules, yellow in life, which form one or more masses in the cell. Surrounding the nucleus is a specialised zone of protoplasm—the perinuclear zone (P.N.Z.)—limited externally by the perinuclear network of neurofibrillæ, which can be clearly seen in the fresh condition; it appears as seen under this magnification as a line (P.N.N.). The peripheral portion of the protoplasm (P.Z.) is pale and non-granular; the general protoplasm contains fine granules, not shown in the figure, smaller than the yellow granules. The sheath of two of the giant cells is shown. × 120.

Fig. 18.—Section of a small giant cell—the fourth cell of the specimen from which fig. 3 (G.C. IV) and the plan shown on p. 450 were drawn. The cell clearly shows the perinuclear, general, and peripheral regions of the protoplasm. Fine fibrils, probably glial, pass from the sheath into the peripheral zone of protoplasm. The perinuclear network of fibrillæ (P.N.N.) is shown, but the general network is not drawn, as it is obscured in the cell by the numerous protoplasmic granules. The details of the sheath are not given, and only about one-third of the thickness of the sheath is represented. × 1000.

Fig. 19.—Section of the nucleus and perinuclear zone of a medium-sized giant cell showing the chromatin granules and achromatic strands of the nucleus, the "double nucleolus" (NL.), the chromophilous granules and the vacuoles of the perinuclear zone of protoplasm, and the perinuclear network of neurofibrillæ (P.N.N.). × 1000.

Fig. 20.—"Double nucleoli" from three giant cells. × 1000.

Fig. 21.—Section of a portion of the peripheral region of a small giant cell in which numerous fibrils, probably of glial nature, are seen passing from the sheath into the peripheral zone of protoplasm, where they branch and interlace. × 1000.

Figs. 22, 23.—Two consecutive sections (each 8 μ thick) of a small giant cell, to show the neurofibrillar network.

Fig. 22 is from a section passing through the nucleus and perinuclear zone. At the margin of the latter is the perinuclear network, continuous on its outer aspect with the general network, which in turn is continuous with the fibrillæ which pass out through the axone. The sheath is too darkly stained to permit details of its structure to be made out. × 1000.

Fig. 23 is a tangential section in which only a small part of the nucleus is seen. The strands of the perinuclear network are distinguished by their slightly greater thickness from those of the general network. × 1000.

Fig. 24.—Section of a large giant cell to show the perinuclear and general network of neurofibrillæ. The origins of the axone fibrillæ do not lie in the plane of this section, but in neighbouring sections these fibrillæ may be traced into connection with the general network. Around the margin of the cell numerous short fibrils—probably glia fibrils (GL.F.)—may be seen passing into the peripheral zone of protoplasm of the cell. Longer entrant fibrils (ENT.F.) appear to join the general network of neurofibrillæ (see pp. 481–483). The perinuclear zone of protoplasm in this cell is extremely narrow; its granules are not shown. Note also the "double nucleolus." × 600.

Fig. 25.—Section of a large giant cell passing tangentially through the perinuclear network to show the stouter strands and closer meshes of this network as compared with the general network. The axone and its fibrillæ are also cut tangentially. Several long fibrils (ENT.F.) enter the cell and apparently become continuous with the neurofibrillar network (see pp. 481–483). Shorter, probably glial, fibrils are also present at the periphery of the cell. Only the inner portion of the sheath of the cell is indicated. × 600.

Fig. 26.—A portion of the perinuclear network of the same cell to show the different levels of the constituent meshes; those nearer the observer are drawn in darker lines. × 1000.

Figs. 27, 28.—Two consecutive sections (8 μ thick) of the nerve cord to show branches of the giant fibres. Nine giant fibres run along the dorsal region of this part of the cord and five run in the middle or ventral portion of the fibrous part of the cord. Three of the dorsal and one of the ventral ones give off branches. The branches of the former fork and the twigs pass into the right and left portions of the neuropile, being lost to view among the ordinary nerve fibres as soon as their sheath disappears (but see pp. 473, 487). BR.$^+$ on fig. 27 is continuous with BR.$^+$ on fig. 28. A portion of BR.$^+$ on fig. 28 is shown more highly magnified in fig. 29. The details of the fibrous part (F.) of the cord are too minute and complex to be represented on this scale of magnification; this area, left white in the figure, consists of interlacing neuroglia fibres and very numerous transverse and longitudinal neurofibrillæ. × 180.

Fig. 29.—A portion of the branch indicated by BR.$^+$ on fig. 28, to show the thin sheath, the neurofibrillæ, and the perifibrillar and interfibrillar substance. × 1200.

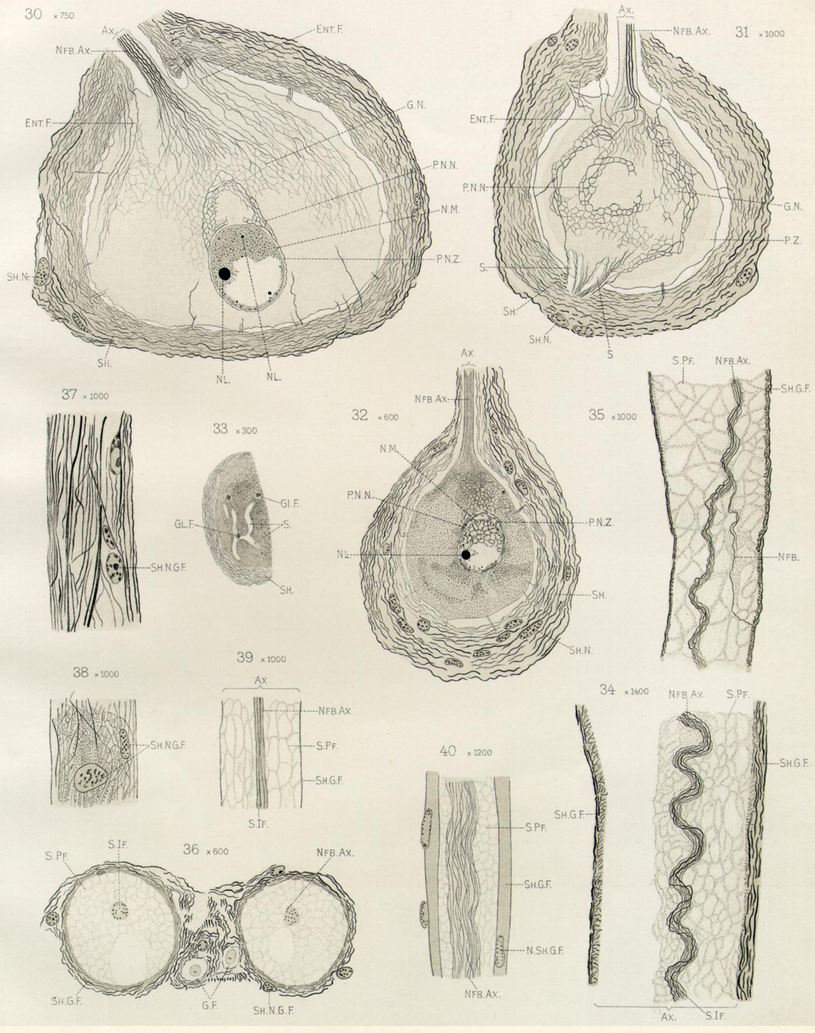


Plate 35.

Figs. 30–36.—*Aglaurides fulgida.*

Fig. 30.—Section of a large giant cell. The perinuclear network is cut tangentially in its upper portion. Each of the axone fibrillæ is formed by the fusion of a number of primitive fibrillæ which are in connection with the general network. This latter, which is composed of very slender strands, is visible over a portion only of the cell. Several long entrant fibrils are seen right and left of the axone. The perinuclear zone is much more oval in shape than the nucleus, and over the greater portion of the nucleus forms only a very thin envelope; the granules of this zone and of the general cytoplasm are not represented (for these, see fig. 32). The large and small nucleoli are added from the next section. × 750.

Fig. 31.—A somewhat tangential section of a medium-sized giant cell. The general network is here differentiated in a zonary manner, there being a zone in which the fibrils are distinctly stouter than the rest and resemble those of the perinuclear network. Sinuses (S.) enter the cell in its lower (ventral) portion accompanied by clusters of fibrillæ, some, at least, of which are glial. Longer entrant fibrils are seen, especially near the axone, some of which appear to be continuous with the neurofibrillar network. × 1000.

Fig. 32.—Section of a giant cell similar to the preceding to show the structure of the nucleus and cytoplasm. The peripheral zone of protoplasm is very feebly differentiated and only over a portion of the cell (*cf.* fig. 18). A darkly stained area extends from the upper end of the nucleus into the cone of origin of the axone. The perinuclear zone of protoplasm and the perinuclear network are indicated, as also are the fibrillæ in the axone; but the general network is not shown, being obscured by the cytoplasmic granules. × 600.

Fig. 33.—Tangential section of a giant cell showing sinuses in the peripheral portion of the protoplasm of the cell. × 300.

Fig. 34.—Vertical section of a large giant fibre showing the sinuous bundle of neurofibrillæ. There are altogether about sixteen fibrils in this bundle, but only seven of these, which are seen on focussing the upper edge of the bundle, are drawn. Two of the fibrillæ are distinctly stouter than the rest. Between the fibrillæ there is a faintly stained, almost homogeneous interfibrillar substance; between the bundle of fibrillæ and the sheath of the giant fibre lies the highly vacuolated, finely granular, perifibrillar substance. × 1400.

Fig. 35.—Vertical section of another giant fibre showing the sinuous bundle of neurofibrillæ from which a fibril crosses the lumen of the fibre to pass out through the sheath. × 1000.

Fig. 36.—Transverse section of the mid-dorsal region of the nerve cord showing two large and two small giant fibres. In each of the two large fibres there are eighteen neurofibrillæ, the cut ends of which are seen lying in the interfibrillar matrix; around this is the vacuolated perifibrillar substance. In each of the two smaller giant fibres there are apparently only two neurofibrillæ. × 600.

Figs. 37–40.—*Halla parthenopeia.*

Figs. 37, 38, 39.—Horizontal sections of a medium-sized giant fibre at three different levels.

Fig. 37.—Tangential section through the outer portion of the sheath, to show that this portion of the sheath is composed of stout, chiefly longitudinal, glia fibrils. Three glia nuclei are also seen. × 1000.

Fig. 38.—Tangential section through the inner portion of the sheath to show the more slender interlacing glia fibrils which form this part of the sheath. The granular remains of the glia protoplasm and two glia nuclei are also shown. × 1000.

Fig. 39.—Central section of the giant fibre showing the slender bundle of neurofibrillæ, the interfibrillar (S.If.), and perifibrillar (S.Pf.) substance. The neurofibrillæ are almost straight (*cf.* figs. 34, 35), as the specimen from which they are drawn was killed fully extended. There are altogether eight to ten fibrillæ in this bundle, but only those are drawn which are seen on focussing the upper edge of the bundle; the other four to six fibrillæ lie at a deeper level. The bundle of fibrillæ is very slender in this fibre, probably owing to the undue contraction of the interfibrillar substance on fixation. × 1000.

Fig. 40.—Horizontal section of a giant fibre. The fibrillæ are very thin and sinuous. The details of the sheath are not shown. × 1200.

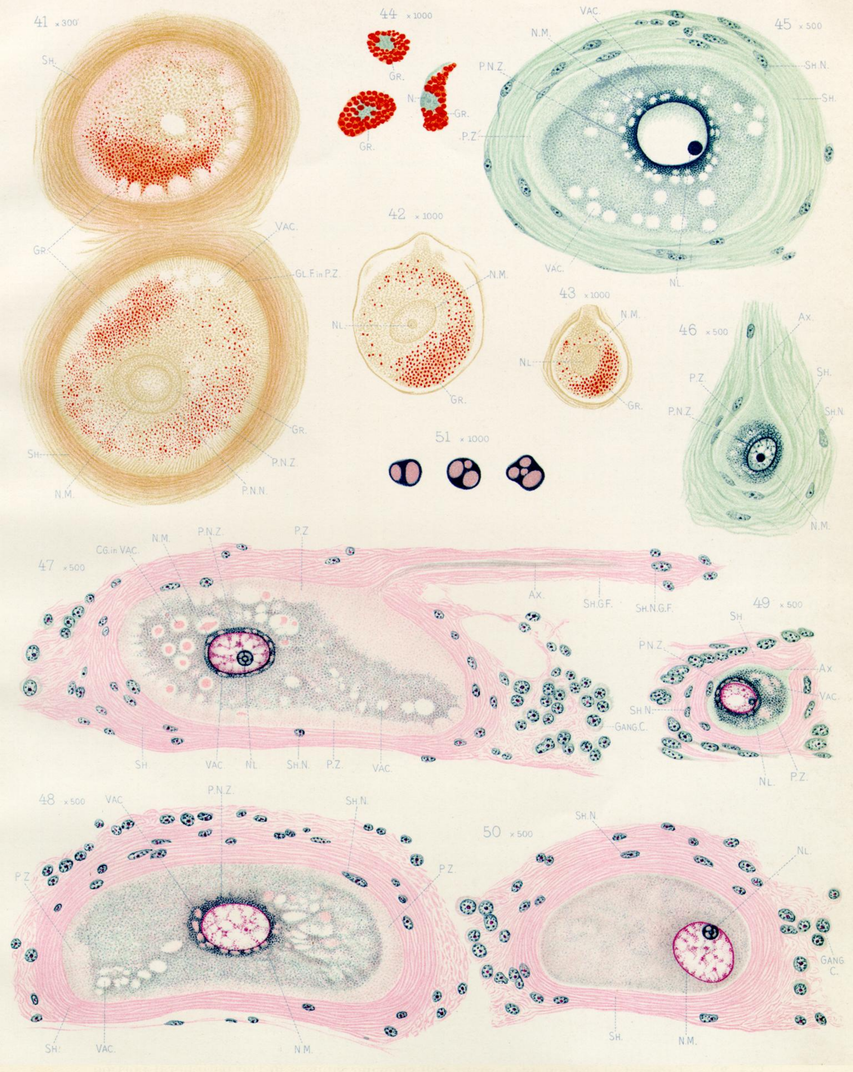


PLATE 36.

Halla parthenopeia.

The figures on this plate were drawn, from preparations by the author, by MR. RICHARD MUIR.

Fig. 41.—Horizontal section of two large adjacent giant cells—a segmental couple. The section from which these are drawn was cut by a freezing microtome from a specimen recently preserved in formalin, and was then stained with Sudan III. The granules upon which this stain and Scharlach R exercise a selective action are shown. They are yellow in the living cell (see fig. 17). The lower cell shows also the nucleus, the perinuclear zone, and numerous fibrils, probably glial, passing from the sheath into the peripheral zone. × 300.

Fig. 42.—A large ganglion cell from the same section as the preceding, showing the granules in the protoplasm, stained with Sudan, chiefly at the end of the cell opposite the axone. × 1000.

Fig. 43.—A medium-sized ganglion cell from the same section as the two preceding figures, showing the granules, stained with Sudan, at the end of the cell opposite the axone. The granules shown in this and in the preceding cell are yellow in life. × 1000.

Fig. 44.—Three small chlorogogen cells from the cellular part of the nerve cord. The section from which these were drawn was cut by a freezing microtome from a specimen recently preserved in formalin, and was then stained successively with Scharlach R and with hæmatein. The cells are full of red-stained granules, most of which were yellow in life. × 1000.

Fig. 45.—Section of a large giant cell stained with methylene blue to show the chromophilous perinuclear zone of protoplasm (the portion of which applied to the nuclear membrane is especially dense), the granular general cytoplasm, and the non-granular peripheral cytoplasm. Vacuoles are present in the perinuclear and general cytoplasm. This method of staining does not show the fibrils of the sheath of the cell, but the glia nuclei of this sheath are well seen. × 500.

Fig. 46.—Section of a small giant cell stained with methylene blue. The perinuclear zone is not so clearly differentiated as in the preceding cell, but the peripheral zone is clearly marked. The cone of origin of the axone is non-granular. × 500.

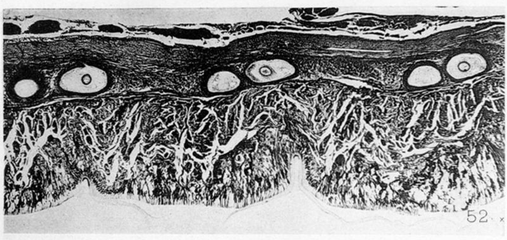
Fig. 47.—Section of a large giant cell stained with toluidin blue and erythrosin. The nuclear membrane and chromatin are acidophile, the granules in the perinuclear zone are basophile, as are most of those in the general cytoplasm. The peripheral zone is stained pink, and the granules therein present are sparse and minute. In some of the vacuoles in the perinuclear and general cytoplasm there is a red-stained coagulum which is granular in some cases. The perinuclear zone in this cell is very narrow and its margin entire (*cf.* fig. 48). Note also the "double nucleolus." × 500.

Fig. 48.—Section of a large giant cell stained with toluidin blue and erythrosin. The perinuclear zone is broader than in the preceding cell and at one end there are strands which extend from it into the general cytoplasm. × 500.

Fig. 49.—Section of a small giant cell stained with toluidin blue and erythrosin showing the protoplasm differentiated into the three well-marked regions—perinuclear, general, and peripheral. The nuclear membrane and chromatin are acidophile, the granules of the perinuclear and most of those of the general cytoplasm are basophile. The peripheral zone and axone are stained faintly blue and are non-granular. × 500.

Fig. 50.—Medium sized giant cell. This and the one drawn in fig. 47 are from the same worm and are stained in the same way, that is, with toluidin blue and erythrosin. The cytoplasm appears, at first sight, to be almost homogeneous, but contains faintly staining granules which are less basophile than usual. There is no definite perinuclear zone and no peripheral zone of protoplasm is differentiated. The nuclear membrane and chromatin are acidophile, as usual. Note also the "double nucleolus." × 500.

Fig. 51.—"Double nucleoli" from three giant cells stained with toluidin blue and erythrosin. Each nucleolus contains acidophile bodies enveloped by a basophile substance. × 1000.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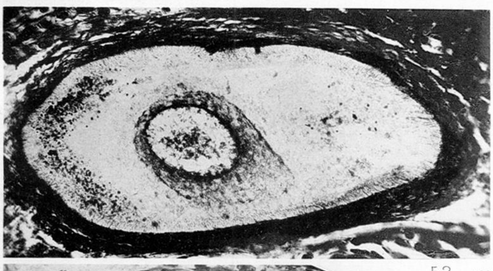
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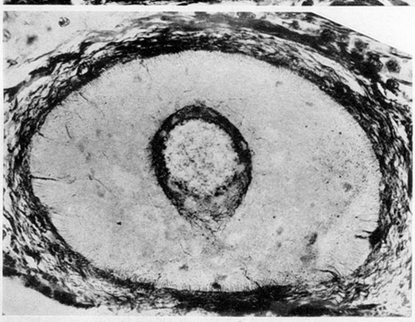
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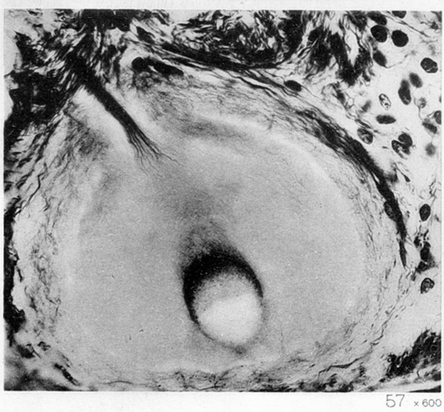
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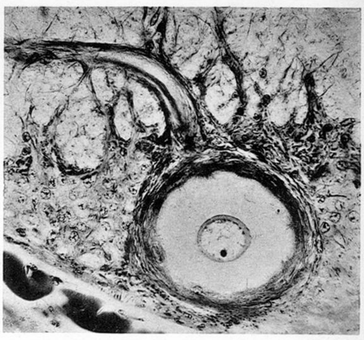
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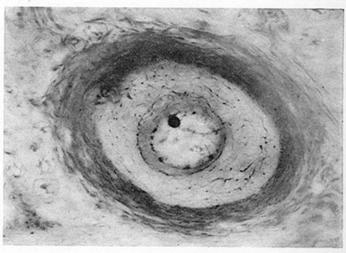
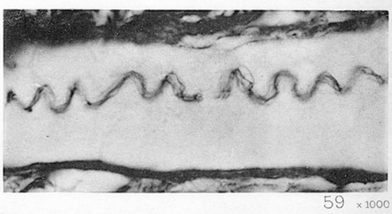

56 × 800


59 × 1000

58 × 1000

PLATE 37.

For the figures on this Plate, which are from untouched negatives, I am indebted to the skill of Mr. MAX POSER (figs. 54–59) and Mr. RICHARD MUIR (figs. 52, 53).

Figs. 52–56.—*Halla parthenopeia.*

Fig. 52.—Vertical section through the ventral body wall and nerve cord. The left side of the section is anterior. The third and fourth and a portion of the second chætigerous segments are included. The section shows the second, third, and fourth couples of primary giant cells, which are so situated that the posterior cell of each couple lies at the posterior end of the segment. The first part of the axone of the second cell from the left is seen. The fourth cell from the left is shown in the following figure. The division of the cord into a dorsal fibrous portion and a ventral cellular portion, the nuclei of which are seen, may be noted. Dorsal to the cord is its thick sheath containing blood-vessels. × 60.

Fig. 53.—The fourth cell from the left in the preceding figure. A large giant cell showing the nucleus, the ovoid perinuclear zone, somewhat more diffuse than usual, and the collection of dark granules in the protoplasm at the anterior (left) end of the cell. The radial lines in the peripheral zone of protoplasm are short glia fibrils. × 480.

Fig. 54.—Another large giant cell from the same specimen showing the perinuclear zone, at the lower margin of which a portion of the perinuclear network is seen. To the right of the upper margin of the perinuclear zone a few strands of the general neurofibrillar network may be seen. Numerous short faintly-stained radial glia fibrillæ are seen passing into the peripheral zone and, on the left, one longer entrant fibril which may be traced nearly to the perinuclear network. × 550.

Fig. 55.—Section of a small, nearly spherical, giant cell and the first portion of its giant fibre to show the course of the latter and its central bundle of darkly-stained fibrillæ. Note also the nucleus and, concentric with it, the narrow perinuclear zone. × 350.

Fig. 56.—Section of a small giant cell showing the nucleus and nucleolus, the perinuclear zone, the perinuclear network and the general network of neurofibrillæ. × 800.

Figs. 57–59.—*Aglaurides fulgida.*

Fig. 57.—Section of a large giant cell and its process to show especially the axone fibrillæ. In the darker cytoplasm to the right of the axone there may be seen slender fibrillæ which have entered through the sheath. The general network, which in this cell is composed of very fine strands, is not seen in the photograph. × 600.

Fig. 58.—The axone and neighbouring portion of the giant cell shown in the preceding figure, showing the axone fibrillæ and, to the right of the latter, entrant fibrils. × 1000.

Fig. 59.—Vertical section of a large giant fibre to show the sinuous bundle of neurofibrillæ lying in the centre of the lumen of the fibre. The sheath of the fibre is clearly seen in the lower (ventral) part of the photograph. Dorsal to the fibrillæ, between them and the sheath of the giant fibre, the vacuolated remains of the perifibrillar substance may be seen. × 1000.